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Review Article

Impact of Leuconostoc Pseudomesenteroides on the Growth Performance of Swiss Albino Mice Administered with Red Powder N (Bakery dye)

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ABSTRACT

Probiotic organisms play a major role in making the intestine a home for several beneficial organisms and a barrier for pathogens. In the present investigation one of the probiotic isolate Leuconostoc pseudomesenteroides was selected by their potentiality. And the red powder N is a food dye a dark red powder with weak typical flavor. It imparts bright and red colour to food stuffs. In this study the mice were divided into four groups with five animals in each group. Here a group one was a control. Group two was administered with Leuconostoc pseudo mesenteroides (1×107 CFU/ml/day) in drinking water daily until 42 days. Group three were supplied with bakery dye red powder N at a dose of 400 mg/kg body weight along with normal diet. Group four were supplied with Leuconostoc pseudomesenteroides(1×107 CFU/ml/day) in drinking water and bakery dye red powder N dose of 400 mg/kg body weight along with normal diet. The animals were observed daily for general health conditions such as body weight, feed conversion ratio, relative weight of organ colon, drinking water consumption and CNS activity of mice. Supplementation of probiotic Leuconostoc pseudomesenteroides in diet through drinking water showed an improvement in the live weight and FCR of mice.

Keywords: Probiotic; Red powder N; Colon; CNS activity; FCR

INTRODUCTION

In recent years there has been a steady increase in community interest towards health promotion and disease prevention. Another one important approach attracting increasing interest amongst consumers and the food industry has been the incorporation of probiotic bacteria into foods [1]. Therefore, the generally recognized as safe (GRAS) status of newly isolated organisms with no previous history to be confirmed by safety evaluation using target animals prior to being incorporated into products [2]. The guidelines for the safety assessment of a novel probiotic strain exist at this stage, and the type of tests that should be included has warranted a much debate16. More recent studies have promoted probiotic specific safety evaluation criteria, especially the infectivity, metabolic activity and immune function of a probiotic strain.

Probiotics are widely applicable in promotion and improvement of health in humans and in animal species. Probiotics have been used as a biologically active substance to a large extent against pathologic conditions ranging from antibiotic-associated with irritable bowel syndrome (IBS), and ulcers due to Helicobacter pylori, hepatic encephalopathy and neonatal necrotizing enterocolitis2. It has been used as a growth, production and promoter of farm animals. There are various scientific reports about the interaction between probiotics and immune system.

Synthetic colors are man-made compounds these are not found in nature, it often azo dyes. Some artificial colors that are used in foods have been linked to negative health issues.Azo dyes are

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generally recalcitrant to biodegradation because of their complex structures, but some microbial consortia or combinations of anaerobic and aerobic systems achieve complete degradation. Lactic acid bacteria (LAB) as promising probiotic isolates could completely metabolize some azo dyes under anaerobic/aerobic regimes. Probiotics are health-promoting live microorganisms that improve the intestinal microbial balance and produce various compounds that inhibit the growth of various bacterial pathogens [3].

The healing process involves the use of probiotics to repair the damaged cells, less epithelial damage can be indeed an efficient way to save energy. However, the use of dietary antibiotics has resulted in common problems such as development of drug-resistant bacteria [4]. Probiotics, could be used as alternatives to antibiotic growth promoters in livestock due to their effects on microflora. Probiotics are defined as viable microbial feed additives which assist in the establishment of an intestinal population which are beneficial to the animal and antagonistic effect to pathogenic microorganisms [5].

The probiotics were screened from the cow's milk and the isolated Leuconostoc pseudomesenteroides strain was given to Swiss albino mice lonely. The impact of the probiotic strain on the mice after challenging them with bakery dye were studied and compared with control groups. The following methods were carried out to assess the ability of probiotics in various aspects of the Swiss albino mice.

MATERIALS AND METHODS

Animals and housing

Male and female Swiss albino mice weighing between 25 – 30 g were used for the study and were housed in a controlled room with a 12 hours light dark cycle and temperature with a humidity of 25 ± 2°C and 55 ± 10 % respectively. They were kept in transparent polypropylene cages and were fed with standard laboratory pellet diet and water at libitum. All the mice were allowed to acclimatize for 7 days to the laboratory conditions before conducting the experiment. The study protocol was approved by Ethical approval was taken from the institutional animal ethical committee (IAEC/prop/2/2018-19) of CPESEA (Committee for the Purpose of Control and Supervision of Experiments on Animals).

Experimental Design

For the research work, the animals were divided into four groups with 5 animals in each group. All the mice were fed with normal diet and two groups were administered with bakery dye red powder N along with the normal diet for 42 days at same doses. Here a group 1 was a control. Group 2 was administered with Leuconostoc pseudomesenteroides(1×107 CFU/ml/day) in drinking water daily until 42 days. Group 3 were supplied with bakery dye red powder N at a dose of 400 mg/kg body weight along with normal diet. The solution of bakery dye red powder N was administered orally, per oral route. Group 4 were supplied with Leuconostoc pseudomesenteroides (1×107 CFU/ml/day) in drinking water and bakery dye red powder N dose of 400 mg/kg body weight along with normal diet.

The animals were observed daily for general health conditions. They were weighed once regularly before feeding the bakery dye red powder N and a daily record of body weight was fostered. After 42 days the animals were forsaken by cervical prolapse. The colons were collected to analyze the toxic effect caused by the bakery dye red powder N.

Chemicals

For the present research work research work bakery dye red powder N was purchased from local market.

LD50 value

The acute toxicity test was conducted to determine the oral LD50 of bakery dye. The 5 mice were randomly selected. The animals were received orally 5 different dosages of dye alone as following (100 mg/kg, 200 mg/kg, 300 mg/kg, 400 mg/kg and 500 mg/kg) while mice in the control group received distilled water. The mice were observed for 42 days for the signs and symptoms of toxicity were recorded [6].

Calculation of LD50

The LD50 of the substances was calculated using the arithmetic method of Karber as modified by Turner (1965)

The LD50 was calculated using the following formula:

LD50 =LDy – Σ (Dd x md)/N

Where LDy =Highest dose (LD100)

N =Number of animals per group

Dd =Dose difference

Md =Mean dead

LD50 =Dose that killed 50% of experimental animals

The mg/kg for bakery dye administration was selected by fixing the lethal dose (LD50). The LD50 value selected for inoculation in the mice 400 mg/kg body weight. Probiotic isolate was serially diluted and plated on MRS medium mice were inoculated with probiotic isolate at a concentration of 1X107 CFU/ml for the assessment of the effects of probiotics on colonization and infection by baker dye.

Body weight and feed conversion ratio

Body weight of individual mice from each group were recorded at weekly intervals using electronic digital balance. Feed consumption was also recorded at weekly intervals and feed conversion ratio FCR was calculated using the following formula,

Relative weight of organs

After 42 days of treatment mice were sacrificed and the organ colon were collected in a petridish and weighed using electronic digital balance, immediately. The colon weight was recorded

after the removal of ingesta. The relative organ weight of individual mice were calculated using the formula,

Relative organ weight = (weight of the organ (gm) / body weight) x 100

Probiotic and bakery dye red powder N with drinking water consumption

Daily probiotic and bakery dye red powder N with drinking water consumption was measured before being offered to mice in each group. Water consumption was measured as difference between the amount of probiotic and bakery dye red powder N with drinking water given and the amount of water left over on daily basis. These were added up at the end of each week to give weekly probiotic and bakery dye red powder N with drinking water consumption values. The weekly consumption value was then divided by the number of mice to obtain weekly probiotic and bakery dye red powder N with drinking water consumption per mice per replicate.

CNS activity of mice

The four groups of animals were marked and weighed. Then the actophotometer was turned on each group of mice and were placed individually in the activity cage for 10 minutes. The basal activity for all the animals were noted. Then bakery dye and probiotics were administered to different groups of animals and after 30 minutes each group of mice was retested for the activity for 10 minutes. The difference in the activity before and after the treatment was noted. From that % change in the CNS activity was calculated.

RESULTS

As indicated in Table 1, it was observed that the body weights of mice after 42 days was significantly higher in the live probiotic culture supplement group 2 (32.43 ± 0.26) when compared to control group 1 (28.34 ± 0.39). In group 3 (bakery dye alone), mice body weight was significantly low (21.71 ±0 .62) as compared to group 4 (bakery dye + probiotics, 26.49 ± 0.52).

Table 1: Changes in the body weight of the mice during 42 days period of study

Treatment	Body weight (g)	
	(Mean ± S.E.M)	
	Initial	Final
Group1	25.82±0.47	28.34±0.39
Group2	26.69±0.33	32.43±0.26
Group3	26.57±0.21	21.71±0.62
Group4	25.98±0.12	26.49±0.52

In the present study the feed conversion ratio in mice on day 7, 21 and 28 were significantly ($P<0.05$) better in all the probiotics supplemented with Group 2 and Group 4 as compared to control group 1 and group 3(Table2). Also on days 35 and 42, the FCR was numerically better in probiotics supplemented (0.78 ± 0.08 and 1.41 ± 0.07) groups when compared to control group1 (0.72 ± 0.03 and 0.91 ± 0.07) and group 3 (0.34 ± 0.05 and 0.53 ± 0.01).

Table 2: Estimation of feed conversion ratio (FCR) in different groups ofmice

FCR (Feed/gm of gain)				
Days	Group1	Group2	Group3	Group4
7	0.38±0.07a	0.32±0.09a	0.36±0.05a	0.38±0.02a
14	0.26±0.02a	0.44±0.01a	0.47±0.01a	0.41±0.06a
21	0.43±0.06a	0.67±0.03b	0.39±0.05b	0.55±0.07b
28	0.56±0.01a	0.72±0.02b	0.67±0.06b	0.63±0.01b
35	0.72±0.03a	0.78±0.08b	0.34±0.05c	0.49±0.03b
42	0.91±0.07a	1.41±0.07c	0.53±0.01c	1.33±0.02c

The findings of the central nervous system on depressant or stimulant properties of mice are presented in Table 3. The mice group 2 with (probiotic alone) showed a CNS stimulant activity due to probiotics. The mice group 4 also showed a CNS stimulant activity of a combination of probiotics and bakery dye. The mice group 3(bakery dye alone) depicted a CNS depressant activity. The CNS activity of probiotic treated group 2 and group 4 showed stimulant activity but bakery dye alone treated group showed depressant activity (group 3).

Table 3: CNS activity of mice before and after the 42 days of study

Treatment	Actophotometer activity (10 minutes)	
	(Mean±S.E.M)	
	Before	After
Group1	406 ± 5	410 ± 7
Group2	523 ± 5	542 ± 8
Group3	461 ± 3	408 ± 6
Group4	441 ± 4	458 ± 6

The present study showed that the colon weight on day 42 was significantly greater for dye treated mice group 3 (1.84 ± 0.26) as compared to control group 1 (1.23 ± 0.12). In group 4 (1.29 ± 0.47) the relative weight of colon remained unaffected by probiotic supplementation (Table.4). The probiotic and bakery dye treated group 4 (1.29 ± 0.47) was attributed to the immunostimulatory status achieved in the colon.

Table4: Average weight of mice colon after the treatment of 42 days

Groups	Colon weight (g)
	(Mean±S.E.M)
Group1	1.23 ± 0.12
Group2	1.27 ± 0.31
Group3	1.84 ± 0.26
Group4	1.29 ± 0.47

The present results of drinking water consumption are presented in Table5. There was no significant difference between the control group 1 (594ml/b) and probiotic administered group 2 (586ml/b) in their water consumption level throughout the experimental period. Whereas mice in dye treated group 3 (692ml/b) the intake level of water was high due to dehydration of water. In group 4 (573ml/b), water dehydration was prevented by probiotic culture S10 supplemented through their drinking water. Water consumption level was not significantly higher. However probiotic treatment prior to the elimination effect of dye in colon indicate the immunostimulatory effect of probiotic S10 on mice performance.

Table 5: Drinking water consumption of different groups of mice

Probiotic and bakery dye red powder N in drinking water for consumption (ml/b/week)				
Days	**Group 1**	**Group 2**	**Group 3**	**Group 4**
7	300	325	418	367
14	423	457	531	443
21	472	461	572	497
28	503	518	596	526
35	529	544	631	555
42	594	586	692	573

DISCUSSION

The results which showed that the acrylamide administration caused marked reduction in mice body weight (30.23 ± 0.22), while those given probiotic supplements along with Acrylamide showed moderate improvement (35.77 ± 0.53) [7]. Similarly reported that by the day of 28, the body weights of all male rats receiving the probiotic strain treatments were significantly ($P < 0.05$) more than the control rats [8].The body weight of stressed mice with probiotic supplementation had a ranged from 0.043 to 0.052. So lactic acid bacteria may have beneficial effects in the intestinal tract and rumen and potentially on moderate rumen conditions thus improving the feed efficiency [9]. A number of workers have reported similarly that reduction in the body weight of rats fed were due to feeding with allura red; in rats fed with some synthetic and natural food colourant and in rats fed with sunset yellow and sodium nitrite and a lack of genotoxic effect of food dyes SY, amaranth and tartrazine and their metabolites when administered along with probiotic twice, at 24 h intervals, by oral gavage in mice [10]. The initial body weight of rats showed no significant difference between groups. After 6 weeks of experimental period, the rats fed with probiotics exhibited an increasing trend in body weight when compared with the control group which fed on with normal diet (441.9 ± 16.3 vs. 415.7 ± 30.1 g) [11].

The FCR in rat recorded that the average weekly feed intake, weight gain and feed conversion ratio in rats fed on the control diet did not differ significantly ($p>0.05$) from the values obtained in rats. Dye treated group 3 mice had lower FCR (0.53 ± 0.01) and lower body weight as compared to all other group of mice. The findings of present investigation indicated that altered absorption of nutrients from gut due to bakery dye treatment resulted in poor growth, poor feed conversion, and lowered body weight. But mice in group 4 (bakery dye + probiotic) had higher body weight and high FCR ratio than those of bakery dye treated non treated group 3[12]. On the contrary the improved FCR were recorded in group II (3.22 ± 0.04) after supplementation with probiotics and caused better utilization of feed ingredients via increased microbial count in goat kids [13]. In group A (normal diet) rats had the highest mean value FCR for at the day of 20 , while group C (iodine treated) had the high value although the difference was not significant ($P>0.05$) [14]. The improved feed conversion in chicken fed a diet supplemented with probiotic. During FCR probiotics had increased the feed intake.The CNS activity could be attributed to the CNS depressant effect of any metabolites in mice and explained that the pentobarbitone (400 mg/kg) produced CNS activity significantly ($P<0.01$) and reduced the onset and prolonged sleep duration, also decreased locomotor activity [15].Any pathological changes in adult mice that was observed CNS demyelination and the pentobarbitone (100 and 200 mg/kg) induced sleeping time test in mice and was found to be highly significant ($p<0.001$) with CNS depressant activity compared to other reference drugs[16]. Dixit and Goyal , (2013) explained that the Indigo carmine dye in swiss albino mice when fed on diet containing 0.0 (control, 0.18g), 0.017 (Low dose, 0.13) and 0.039 (High dose, 0.1) gm per kg body weight of dye for 42 days (6 weeks) showed a significant decrease was observed in the weight of organs. This indicates the anti-androgenic nature of the dye. The weight ratio of the colon in dextran sulfate sodium treated mice (an indicator of colonic edema) was significantly higher in the colitis groups than the control group [17]. The food azo dyes increases the weight of organs in mice (spleen, liver and small intestine) [18] .The entrofloxacin mice treated group had a significant ($P < 0.05$) change in water consumption which during the week increased from 9.7 ± 0.4 mL/d to 11.4 ± 0.2 mL/d per pair of mice and analyzed that any chemically treated mice could be enhanced for water consumption during treatment[19].

$$FCR = \frac{Average\ food\ consumption\ per\ mice\ per\ week\ (gm)}{Average\ weight\ gain\ mice\ per\ week\ (gm)}$$

CONCLUSION

Finally, supplementation of probiotic in diet through drinking water showed an improvement in the live weight and FCR of mice. It was efficient in controlling the effect of dye in colon, consequently improving mice performance. S10could be beneficial in controlling immunosuppressed environmental condition and reducing the infection in mice.

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