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Impacts of Spirulina-Based Probiotic Feed on Swiss Albino Mice and its Effects on Digestive Enzymes

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Abstract: Spirulina is widely used as food because of its rich nutrient contents. They play an important role in gut enzyme activity. The present study determined the digestive tract enzyme activity in spirulina administered Swiss albino mice using a set of biochemical parameters. The experimental group of Swiss albino mice was administered to two different concentrations of spirulina, i.e., 3 and 6% spirulina with 20 days experiment. When the mice were fed with 6% spirulina weight loss has been observed in the experimental animal. Weight loss has been observed about 2.0±0.72 μg mL^{-1} had been noticed when the Swiss albino mice is treated with concentration of 6% spirulina based probiotic feed. By contrast, weight gain of 5.16±2.68 mg was also observed when the experimental mice fed with 3% probiotic food. These results represented the variation in the enzyme activity after the treatment with experimental feed such as amylase level showed 18.9 μg mL^{-1} in the foregut and 11.3 μg mL^{-1} in the hindgut. Lipase level was 45.8 μg mL^{-1} in foregut and 47.8 μg mL^{-1} in hindgut. Catalase activity drastically decreased in mice treated with 6% spirulina and enzyme activity of lipase and catalase activity increased in mice treated with 3% spirulina. This study suggests that the spirulina provided in little amount does not show much variation, whereas the spirulina taken in high concentration shows slight changes in the production of digestive enzymes on the Swiss albino mice.

Key words: Digestive enzymes, gut enzymes, probiotic feed, spirulina, India

INTRODUCTION

Spirulina based probiotic feed has a tremendous importance in nutritional, industrial and environmental biotechnology (Vonshak, 1997; Breman-Craddock *et al*., 1992) and is best known for its high protein content as well as enriched nutrients. Previously, Suneetha *et al.* (2009) depicted that digestive enzymes are biocatalysts; they speed up the rate of chemical reactions taking place in the living cells, especially during the digestion processes. According to Abd El-Baky *et al.* (2007), spirulina contains higher amount of nutrition, hence it is known as the nutritional powerhouse spirulina is a filamentous cyanobacterium which provide antioxidant and anticancer effects, although they are having enzymes, nutrients, lipids and carbohydrates. Ali *et al.* (2008) reported that spirulina possesses different kinds of gut enzymes like lipase, catalase and invertase in the digestive system. It can be readily activated towards the production of enzymes and helpful in extracellular operations such as digestion in the gut region of the organisms (Lone and Nallet, 1985; Maria, 1998). In case of enzymes that breakdown starches is known as amylases and enzymes that digests fat is known as lipases, experimental evidences clearly shows that no organisms could survive without these enzymes (Zoetemdal and Erwin, 2001). Very recently Shiffalli *et al.* (2009) stated that various spirulina species are used as a good food supplement, especially *Spirulina laxissima* act as a food supplement of other mixed probiotic content, including the therapeutic purposes. Accordingly, James *et al.* (2008) determined that spirulina containing the enzyme of invertase hydrolyses the O-glycosyl bond forming an equimolar mixture of glucose and fructose from sucrose and invertase possess wide application in the food and pharmaceutical industry because of its desirable properties that show high solubility and hygroscopic nature. Earlier, Ali *et al.* (2008) described that the spirulina containing nitrate-assimilating enzyme has higher specific activities and are more stable than other food sources. However, there is no other research has been performed in this aspect, hence researchers aimed on following objectives:

- To find out the weight gain and weight loss of spirulina probiotic food treated Swiss albino mice
- To study the influence of spirulina based probiotic feed for enzyme production in Swiss albino mice

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MATERIALS AND METHODS

Probiotic food chosen for the study: This study has been performed in the Department of Biotechnology, Malankara Catholic College, Mariagiri during the year 2009. In India, spirulina is produced primarily as a food supplement and are available in the health food markets. Moreover, recently there has been enormous interest concerning on supplementary effects of spirulina as a probiotic food as well as a booster for the immune system on animals. The commercially available spirulina capsule was bought from a pharmaceutical shop, Kaliakkavilai. The nutritional composition of this spirulina power contains protein, carbohydrate, lipids (protein 60-70%, carbohydrates 15-20%, minerals 7-13% lipids, 6-8% and moisture 8-7%). As spirulina is rich in nutrients, it is selected for this study.

Maintenance of experimental animal: In this experiment, Swiss albino mice were chosen as the representative of environmental species. The mice were kept in an animal house under standard condition of light and darkness (11:13) and temperature were maintained at 32±1.5°C. The mice were fed with standard laboratory mice pellet feeds, consisting protein 60-70%, carbohydrates 15-25% and minerals 7-13%. A total of 15 mice was selected for this study with 30 days old and having the weight of 53.1±3.0. These mice were separated into 3 groups based on the size of the individuals. After these grouping, the mice were kept and maintained in the animal house with specially designed polyethylene cage to provide the systematic supply of food and water.

Preparation of spirulina based probiotic feed: Spirulina capsules was collected from a pharmaceutical shop, Kaliakkavilai. Spirullina is mixed with mice natural feed, described by Abd El-Baky *et al.* (2003), 3% (3 g spirulina powder + 97 g natural food of mice) and 6% (6 g spirulina powder + 94 g natural food of mice).

Experiment on Swiss albino mice: About 30 days old laboratory acclimatized Swiss albino mice weighing 53-54 kg were taken from already categorized group. Each group has been separated and provided daily with two different concentrations (3 and 6%) of food. This experiment was prolonged up to 20 days. The unfed feed and waste matters were removed daily also. A total of 100 mL of water was given to both experimental as well as control Swiss albino mice. Feeding performance and food consumptions were observed throughout the experimental period.

Procedure for enzymes: Foregut and hindgut tissues were collected after 20 days from spirulina administered mice and control mice. Thereafter, the foregut and hindgut were separated from the rest of the alimentary tract and flushed with ice cold physiological saline to make free from other debris and stored in refrigerator at 4°C for 1 day. Both foregut and hindgut tissue homogenate samples were centrifuged at 3000 rpm for 10 min to remove the tissue residues and other impurities from both gut regions. Finally, this homogenates 10% (w/v) of both tissues (fore and hind) content enzymes were analyzed according to Sadasivam and Manickam (1970). The mice were provided with 3 and 6% spirulina mixed with the natural food, for a period of 20 days and weight gain and weight loss of the mice were recorded. After decapitation, the gut of mice were removed, cleaned and immediately fixed in the phosphate buffer saline. The foregut and hindgut tissues were taken separately and the homogenized samples were centrifuged at 3000 rpm for 10 min after removing tissue residues and other impurities from both gut regions. Enzymes like amylase, lipase and catalase were analyzed according to Sadasivam and Manickam (1970).

RESULTS

Initially, when the mice were fed with 3 and 6% concentrated experimental food, researchers have observed the weight gain and weight loss, respectively. When the mice were fed with 6% spirulina weight loss has been observed in the experimental animal. Weight loss has been observed about 2.0±0.72 g when the Swiss albino mice were treated with concentration of 6% spirulina based probiotic feed. By contrast, weight gain of 5.16±2.68 g also has been observed when the experimental mice fed with 3% probiotic food. This results suggest that the concentration of spirulina content increases to three fold level the food consuming rate of Swiss albino mice decreases when compared to food in taken by the control mice (Table 1).

Determination of amylase activity: The amylase activities in mice were assayed according to Sadasivam and Manickam (1970) and the values were expressed in: Moles of starch hydrolyzed/μg/mL on foregut and hindgut of mice, respectively. This enzyme activity was observed in

Table 1: Weight gain and loss of Swiss albino mice treated with spirulina based probiotic food

Spirulina provided (%)	Initial weight of mice (g)	Final weight of mice (g)	Weight gain (g)	Weight loss (g)
3	53.7±5.10	59.80±2.0	5.1±2.68	-
6	53.1±3.00	39.00±5.2	-	2.0±0.35
Control	54.2±0.74	98.44±3.7	8.42	-

foregut tissue of treated mice provided with 3 and 6% spirulina based probiotic supplementary feed in 30 days old mice are shown in Table 2. The 3% spirulina treated mice possessed the maximum amylase activity in the foregut (27.5 starch hydrolyzed/μg/mL categories of the Swiss albino mice). The second most peak activity was noted in hindgut (19.7 starch hydrolyzed/μg/mL) of 3% treated mice; lower amylase activity on foregut and hindgut enzyme activity also decreases. The overall results shows that the foregut of the treated mice has the maximum amylase secretion than the hindgut region of both spirulina treated and control mice.

Determination of lipase activity: Another study made on the enzyme activity in mice is lipase. Mice treated with 3% spirulina did not show many variations in the foregut (55.9 fatty acids liberated/min/μg/mL) and hindgut (45.3 fatty acids liberated/min/μg/mL) and the activity has been observed in hindgut than in the foregut region of mice treated with 6% spirulina (Table 3). When comparative study has been made between the control and spirulina (6%) fed mice, individuals shows that the spirulina provided mice shows lowest lipase activity than the control mice. From these results, it is clear that the maximum influence of lipase activity had been observed in the hindgut than in the foregut region.

Determination of catalase activity: In case of catalase enzyme on spirulina treated mice, maximum catalase activity was observed in foregut (31.8 μg mL^{-1}) region of mice treated with 3% spirulina. At the same time minimum 11.7 μg mL^{-1} of catalase had been noted in the hindgut region, concentration of 6% spirulina treated mice shows maximum and minimum catalase activity of 11.7 and 31.8 μg mL^{-1} on foregut and hindgut region, respectively (Table 2). Among the three kinds of digestive enzymes, it was clearly showed the catalase activity was maximum in foregut than that of hindgut region.

The amylase activity in the foregut after the treatment with probiotic feed is mentioned in Table 2. Maximum amylase activity was observed in foregut 27.5/starch hydrolyzed/μg/mL observed compared with control. Moreover, similar enzyme activity showed in hindgut too. Furthermore, amylase activity was higher 19.7 starch hydrolyzed/μg/mL treated with 3%, although the amylase activity was observed lowest amount 11.37 starch hydrolyze/mL compared with 6% treated spirulina. This result clearly shows the decreased enzyme activity of both foregut and hindgut; however, foregut of the treated mice has shown maximum amylase secretion than the hindgut region of both spirulina treated and control mice.

Table 2: Enzymes in the foregut region of spirulina administered Swiss albino mice

Name of the enzyme (g mL^{-1})	(3%)	(6%)	Control
Amylase	27.5±1.23	18.9±0.68	46.8±1.47
Lipase	55.9±2.33	45.8±0.79	51.0±1.37
Catalase	31.8±2.47	11.7±0.73	28.4±0.58

Table 3: Enzymes in the hindgut region of spirulina administered Swiss albino mice and control

Name of the enzyme (g mL^{-1})	(3%)	(6%)	Control
Amylase	19.7±1.25	11.3±1.34	39.1±2.61
Lipase	56.5±2.73	47.8±0.19	53.0±2.44
Catalase	21.5±2.61	9.8±2.54	38.0±2.54

DISCUSSION

Enzymes are protein that catalyzes chemical reactions. The digestion is not possible without the activity of the enzyme such as amylase, protease and lipase and catalase in mice which break down and helps to build substances that body need in the case the spirulina based probiotic feed. The results of the present study shows that when the concentration of spirulina based probiotic food increased in mice, the influence of enzyme activities such as amylase, lipase and catalase activity also varies, remarkable decrease in the activity of the enzymes in the hindgut region was observed. This may be due to the fact that the higher consumption of spirulina leads to fast movement of food through the foregut and hindgut, respectively.

When the mice fed with higher concentration of spirulina, it has become alkaline in nature. In this condition, the stimulation of natural enzymes was detoxified in the mice. The present results are further confirmed by the observation of Pillai *et al.* (2009) as spirulina acts as antioxidant and detoxifying the enzyme though the spirulina based probiotic food improves cell nucleus enzyme activity and DNA synthesis. Many researchers stated that when spirulina was taken on a regular basis, the metabolic activities are almost turning in an irregular way. Present findings shows that depending upon the increased irregular content of spirulina could be leads to physiological upset earlier (Maria, 1998; Abd El-Baky *et al.*, 2003; Prem *et al.*, 2004). Furthermore, Ali *et al.* (2008) reported that when the complete protein was consumed, digestion difficulties can be prevented from the assimilation of all needed elements which provides all the required amino acids and that is 5 times easier to digest than that of the other probiotic feeds. Few aggregated postulates made between other living organisms, predicted that spirulina probiotic feed stimulates the production of enzyme that transport fats within the organisms.

Furthermore, Rao *et al.* (1996) and Genene (2009) stated that spirulina food can easily be digestible which liberating considerable energy has shown to be 83-95% and amino acid balance is around 90%. Rao *et al.* (1996) and Genene (2009) documented that spirulina consisted food accomplished with the metabolically active enzymatic process from the beginning to end including physiological mechanisms. Hence, this spirulina based probiotic feed mainly influenced both hindgut as well as foregut enzyme activities during the digestion processes. Recent studies have found that spirulina algae functions as probiotic by allowing the immune system to function with higher metabolic activities. Enzymes present in spirulina are converted into simple molecules of food. It can withstand in relatively high temperatures without losing its properties (Amrisha and Ganguly, 1990; Ali *et al.*, 2008). Activation of enzymatic activity of catalase, amylase and lipase in alimentary canal shows variations. The variation has been observed in catalase activity in the control mice and mice treated with spirulina in different concentrations. This type of similar observations were already been reported by Kirkman*et al.* (1987) and Alymi and Eman (1998). Catalase is a common enzyme found in all the living organisms which are exposed to oxygen when it functions to catalyze the decomposition of hydrogen peroxide to water and oxygen (Hayashi *et al.*, 1998). It contains four porphyry (iron) groups that allow the enzyme to react with the hydrogen peroxide. From this experiment, researchers conclude that when the concentrations of spirulina increases then the catalase, amylase and lipase enzymes in gut region were decreased which may be due to several enzymatic and non-enzymatic antioxidant defense mechanisms to maintain the concentration of spirulina based probiotic by this similar supportive evidence published by Gloerich *et al.* (2005). Modification of the activity of digestive enzyme in mice have related to the quantity and quality of some components in the diet (Abd El-Baky, 2004). The present results are further confirmed by the observations of Vijayakumar *et al.* (2000) and Ronald and Richard (2009) on nutritional ability and it influences the digestive enzyme activity. The result of the present investigation clearly shows maximum activity of amylase, lipase and catalase in foregut tissue when compared with the hindgut based upon the concentration of probiotic feed, as the foregut tissue is the chief site for the secretion of the digestive enzyme.

CONCLUSION

The intent of this study was to examine the spirulina based probiotic feed and its influence in digestive enzymes (amylase, lipases and catalase). The amylase activity is evoked by spirulina based probiotic feed at the concentration of 3% (27.5/starch hydrolysed/µg/mL) the next peak amylase activity was noted on hindgut region (19.7/starch hydrolysed/µg/mL. About 6% spirulina treated mice showed minimum enzyme activity on the foregut and hindgut digestive enzymes such as lipase, amylase and catalase. However, these 3 digestive enzymes, i.e., amylase, catalase and lipase clearly shows that foregut region has higher enzyme activity than the hindgut region except the lipase enzyme. This study concluded that when the spirulina based probiotic feed was provided in high concentration the enzyme activity of amylase, lipase and catalase declines in Swiss albino mice. When the mice fed with spirulina based probiotic feed enzyme activity alters the metabolic system it can also modified the antioxidant activity as well as detoxifying activity in mice.

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