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Nutraceutical Effects of Fermented Whey on the Intestinal and Immune System of Healthy Albino Rats

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Abstract: The effects of oral administration of different volumes (0.5, 1.0, 1.5, 2.0 and 2.5 mL) of fermented whey on the immune system of healthy albino rats were investigated. The immunostimulatory effect of the fermented whey was tested by evaluating the White Blood Cells (WBC), Packed Cell Volume (PCV) and the total differential WBC counts. Twenty four rats divided into six groups of four rats per group were used. Each group was fed with different volume of whey along with basal diet while the group that serves as control was given basal diet only. Heamatological assay was carried out by collecting the rats' blood through cervical dislocation into EDTA bottles and then analyzed. The initial weights of the rats and changes in weight were monitored daily. The administration of whey to the healthy rats shows that there was boosting of the immune system. This was evident in the increase in the values of PCV and lymphocyte counts of the groups fed with whey as compared with control. The results of serum enzymes also reveal that there was no sign of toxicological defect on the internal organs when administered to healthy rats. It is therefore conceivable that the consumption of whey by apparently healthy individuals will boost their immune system and would not constitute any hazard to the internal organs.

Key words: Whey, nutraceutical, internal organ, immune system

INTRODUCTION

Nutraceutical refers to extracts of foods claimed to have a medicinal effect on human. More rigorously, nutraceutical implies that the extract or food is demonstrated to have a physiological benefit or provide protection against a chronic disease (Health Canada, 2002). Today the exploration and exploitation of the disease-fighting properties of a multitude of phytochemicals found in both food and non-food items have created a renaissance in human health and nutrition research. At the same time, many opportunities for the development of novel dietary products have been created (Beecher, 1999). Staying healthy has an advantage. Proper functioning of the immune system constitutes a great deal too staying health or total health of the body. The immune system consists of factors that provide innate and acquired immunity and has evolved to become more specific, complex, efficient and regulated. One of the principal functions of the human immune system is to defend the body against infecting and other foreign agents by distinguishing self from non-self (foreign antigens) and to marshal protective responses from leukocytes. The immune system, if deregulated, can react to self-antigens to cause autoimmune diseases or fail to defend against infections. The immune system is organized into discrete compartments to provide the milieu for the development and maintenance of effective immunity.

Consumers are increasingly interested in the health benefits of foods and have begun to look beyond the basic nutritional benefits of food to the disease -prevention and health- enhancing compounds contained in many foods. This combined with a more widespread understanding of how diet affects disease, health-care costs and an aging population has created a market for functional foods and natural health products.

In the investigations carried out by Gustavo *et al.* (1989) they observed that minor changes in the amino acid profile of a defined formula diet can influence the immune response without having any significant effect on the nutritional status of the host. Counous (2000) went further to investigate the presence of amino acid in whey. He came up with the report that whey contains cysteine, which raises glutathione level. Glutathione is powerful antioxidants with the ability to help the body reduce the risk of infections. Olorunfemi *et al.* (2006, 2007) also carried out an investigation to determine whether any benefit may be derive from whey which has been aforetime considered a waste in Nigeria. They reported that whey fermented for 72 h has antidiarrhoeal potential.

In this present study the effect of fermented whey on the internal organs and immune system of healthy albino rats was investigated.

MATERIALS AND METHODS

Fresh whey sample was obtained from local whey manufacturing point at Akure-Owo express road, Akure. The fresh samples were collected in separate sterile container and were transported immediately to the laboratory for investigation.

Twenty four Wister strain albino rats (*Rattus novegicus*) aged between 6-8 weeks old used for the investigation was purchased from Biochemistry Department, University of Ilorin, Kwara State. They were divided into groups of four rats per group. During the period of the experiment the rats were kept on water and growers mash. The feeding experiment was performed at the animal house of the Department of Animal Production and Health, Federal University of Technology, Akure. A seven days acclimatization period was observed before the commencement of the experiments.

Effect of Feeding Healthy Rats with Whey on their Performance

The initial weights of rats, weight gained within five days and the final weight gains were recorded for healthy rats. The groups of rat were fed with different volumes of fermented whey (0.5, 1.0, 1.5, 2.0 and 2.5 mL) along with the mash. The rats were killed by cervical dislocation and their blood samples were collected into EDTA bottles for haematological parameters and serum enzymes analyses.

Analysis of Serum Enzymes

Serum Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assayed by the method of Reitman and Frankel. The Bessy Lowry and Brock alkali phosphate method was used for the analysis of serum alkaline phosphase.

RESULTS AND DISCUSSION

The results of the haematological studies shown in Table 2 revealed that whey improved the health condition of the infected rats by boosting their immunity. This was evidence by the significant ($p<0.05$) increase recorded in the values of Packed Cell Volume (PCV) and White Blood Cell (WBC) levels of the healthy rats dosed with different volumes

Table 1: Haematological parameters of healthy albino rats before whey administration

Treatment (Vol. of whey)	PCV (%)	WBC $\times 10^9$ L^{-1}	Neutrophils	Lymphocytes	Monocytes	Eosinophils
			(%)			
0.5	30.25±0.89^{d}	2.56±0.43^{b}	48.25±1.14^{b}	50.75±0.39^{c}	1.00±0.00^{b}	0.00±0.00^{a}
1.0	28.25±1.08^{b}	2.53±0.23^{b}	49.00±0.51^{b}	50.00±2.04^{c}	0.25±0.50^{a}	0.00±0.00^{a}
1.5	26.15±1.63^{a}	2.75±0.14^{c}	49.00±0.35^{b}	49.75±0.20^{bc}	1.00±0.00^{b}	0.25±0.50^{a}
2.0	29.75±1.08^{c}	2.10±0.19^{a}	58.25±0.78^{cd}	40.75±0.37^{a}	0.50±0.57^{a}	0.00±0.00^{a}
2.5	28.25±0.91^{b}	3.01±0.44^{c}	56.50±1.47^{c}	42.50±0.73^{b}	0.00±0.00^{a}	0.00±0.00^{a}
Control	26.05±0.61^{a}	2.80±0.22^{c}	43.50±0.93^{a}	55.50±0.36^{d}	0.00±0.00^{a}	1.00±0.00^{b}

Control: Rats without whey. PCV: Packed cell volume; WBC: White blood cell. Values are mean of four replicates±SD. Values followed by similar alphabets along the same column are not significantly different ($p<0.05$)

Table 2: Effect of administration of whey on the haematological parameters of healthy albino rats

Treatment (Vol. of whey)	PCV (%)	WBC $\times 10^9$ L^{-1}	Neutrophils	Lymphocytes	Monocytes	Eosinophils
			(%)			
0.5	32.25±0.89^{a}	4.56±0.43^{c}	47.25±1.14^{b}	51.75±0.39^{a}	1.00±0.00^{b}	0.00±0.00^{a}
1.0	30.25±1.08^{b}	4.53±0.23^{c}	43.00±0.51^{b}	56.00±2.04^{a}	0.25±0.50^{a}	0.00±0.00^{a}
1.5	33.75±1.63^{c}	3.75±0.14^{b}	42.00±0.35^{ab}	56.75±0.20^{ab}	1.00±0.00^{b}	0.25±0.50^{a}
2.0	32.75±1.08^{c}	3.10±0.19^{a}	41.25±0.78^{a}	57.75±0.37^{b}	0.50±0.57^{a}	0.00±0.00^{a}
2.5	30.75±0.91^{b}	4.35±0.44^{c}	41.50±1.47^{a}	57.50±0.73^{b}	0.00±0.00^{a}	0.00±0.00^{a}
Control	26.75±0.61^{a}	2.80±0.22^{a}	43.50±0.93^{b}	55.50±0.36^{a}	0.00±0.00^{a}	1.00±0.00^{b}

Control: Rats without whey. PCV: Packed cell volume; WBC: White blood cell. Values are mean of four replicates±SD. Values followed by similar alphabets along the same column are not significantly different ($p<0.05$)

of whey. The significance increase in the haematological parameters of the rats fed with whey was noticeable when the values of these parameters were compared to those without whey (Table 1). Increase in PCV showed that whey causes an improvement in the volume of blood. An increase in PCV might have increase the amount of nutrient the red blood cell can circulate which in turn may be responsible for the increase in the body weight. A high level of PCV is an indication that the rats were not anaemic while a lower level indicates anaemic condition (Schalm *et al*., 1975). There was also an increase in the WBC. White blood cells especially the lymphocytes are of great importance in defending the body against infection (Schalm *et al*., 1975). The lymphocytes in the groups fed with 2.5, 2.0, 1.5 and 1.0 mL^{-1} of fermented whey significantly increased from 42.5, 40.75, 49.75, 50.0 to 57.50, 57.75, 56.755 and 56.00%, respectively, after been fed with whey The result of the lymphocyte count of the control experiment showed no significant difference.

The safety of whey for human consumption were verified using histopathological analysis and serum biochemical markers such as AST, ALT, ALP, total protein and globulin. The toxicological assessments results in Table 3 revealed that there were no significant increases in the level of all the biomakers such as those obtained for the control experiment. An increase in the blood AST, ALT and ALP has been reported as an indication to both liver damage and possible damage to the heart (American Liver Foundation, 1999; Johnston, 1999; Oyetayo and Osho, 2004; Agarry and Osho, 2005). Meanwhile, from the result obtained, it could be established that whey has no toxic effect on the liver and the heart since no increase was observed in the values obtained. Kelly (1997) also reported that there are no side effects in whey protein with proper supplementation.

In affected liver, both the ALT and ALP levels will be increase (Strove, 1989). The ALP values indicate to the biliary system, either within the liver or in the larger bile channels outside the liver. The ALP is elevated in a large number of disorders that affect the drainage of bile, such as gallstone or tumor blocking the common bile duct. It serves as an indicator of liver damage when there is cholestasis or lack of bile flow (Johnston, 1999). The AST and ALT are enzymes that are located in the liver cells and leak out and make their way into the

Table 3: Effect of the administration of whey on the biochemical indices of albino rat serum

Groups	AST (iμ L^{-1})	ALP (iμ L^{-1})	ALT (iμ L^{-1})
Control	64.47±0.35^{b}	56.32±0.49^{a}	17.16±4.89^{a}
Group A	63.94±0.12^{a}	56.45±0.12^{a}	16.84±0.10^{a}
Group B	64.44±0.20^{b}	56.12±1.63^{a}	17.22±7.52^{a}

Group A: Group of four rats orogastrically fed with 2.0 mL of whey, Group B: Group of four rats orogastrically fed with 2.5 mL of whey, Control: Group of four rat without whey. AST: Aspartate-aminotranferase, ALT: Alanine-aminotranferase, ALP: Alkaline-phosphatase. Values are mean of four replicates±standard deviation. Values followed by similar lette(s) along the same column are not significantly different ($p<0.05$)

Table 4: Effect of the administration of whey on the albino rat serum protein

Groups	Albumin (g dL^{-1})	Globulin (g dL^{-1})	Protein (g dL^{-1})
Control	4.36±0.00^{ab}	2.72±0.00^{a}	7.08±0.12^{a}
Group A	4.43±0.13^{b}	2.66±0.14^{a}	7.09±0.55^{a}
Group B	4.30±0.10^{a}	2.73±0.13^{a}	7.03±0.17^{a}

Group A: Group of four rats orogastrically fed with 2.0 mL of whey. Group B: Group of four rats orogastrically fed with 2.5 mL of whey. Control: Group of four rat without whey. Values are mean of four replicates±standard deviation. Values followed by similar alphabets along the same column are not significantly different ($p<0.05$)

general circulation when the liver cells are injured (American Liver Foundation, 1999; Johnston, 1999). The ALP is regarded to be a more specific indicator of liver inflammation, since AST may be elevated in diseases of other organs such as heart or muscle disease. In chronic hepatitis or cirrhosis, the elevation of these enzymes may be minimal and less than 2-3 times normal or moderate 100-300 μ L^{-1}. Mild or moderate elevations of ALT and AST are non-specific and may be cause by a wide range of liver diseases. AST is widely distributed in body tissues; hence many other diseases involving cellular injury may be accompanied by increases in AST activity for example, severe bacterial infection, malaria, pneumonia, pulmonary infections and tumor (Chessbrough, 2004).

Albumin is produced mainly in the liver and its estimated is a test of liver function (Johnston, 1999). The albumin levels of rats fed with whey were not significantly different ($p<0.05$) from those of the control. This showed that whey did not increase the biosynthesis of albumin from the liver (Table 4).

To date, no severe adverse reactions have been noted following administration of whey although many individuals sensitive to dairy find that casein is the culprit and they can tolerate whey. Generally, the result of the toxicological assessment revealed that whey contains no toxic substances that may be hazardous to health and its administration improves the health of infected animals.

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