

## A Comparative Study of Egyptian Dairy Products on Biochemical, Immunological and Histological Indices of Animal Models

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**Abstract:** This is a comparative study between different dairy products fed at 10% of rat's diet. A series of immunological, histological, bacteriological and biochemical parameters were carried out. The rats which were fed on buffalo colostrum diets showed higher levels of serum immunoglobulin, an improvement of liver functions, histology of colon and liver tissues and lower percentage of body weight gain compared with other diet groups. Meanwhile the fermented milk diet showed the least improvement compared with the control group. Surprisingly no *bifidobacteria* was found in fermented milk supplemented with probiotic although the labels on the product indicated the presence of it.

**Key words:** Colostrum • dairy products • rats • histology • ELISA • liver functions

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### INTRODUCTION

Nutritional status has a major impact on the immune system and the overall wellbeing. Mediterranean diets, fermented milk and probiotics are assumed to have a favorable effect on intestinal microflora and human health. The high incidence of chronic diseases such as cancer, atherosclerosis, rheumatoid arthritis and irritable bowel syndrome is of a major concern to health professionals and the governments who are continually striving to reduce the occurrence and consequences of such diseases [1]. Modern life style implies a reduced intake of beneficial bacteria; specifically in Western diets. In Egyptian diets, the situation could be far from previous in terms of quality of meals, dairy products (fermented milk) consumption, whole grain, legumes, fruits and vegetable contents.

The increasing interests in a healthy diet is stimulating innovative developments of scientific products in the food industry. The viable lactic acid bacteria in fermented milk products, such as yoghurt, have been associated with an increased lactose tolerance, well-balanced intestinal microflora, antimicrobial activity, stimulation of the immune system and anti-tumoural, anti-cholesterolaemic and antioxidative properties in human subjects [2, 3].

Dairy products are foods rich in many nutrients that may be enhancing health and beneficial in preventing

different diseases. In terms of health it plays a role in promoting normal growth and healthy bones and teeth. For diseases it has been known to lower blood pressure and reduce the risk of high blood pressure, particularly when included as part of a dietary pattern that is low in fat and rich in fruits and vegetables. A critical review on the Dietary Approaches to Stop Hypertension (DASH) diet concluded that health professionals should focus on dietary patterns rather than individual nutrients that can effectively lower blood pressure [4]. Dairy products are sources of at least four different minerals/electrolytes that can affect blood pressure, bone and teeth health (i.e. calcium, sodium, potassium and magnesium) [5].

Colostrum is the pre-milk fluid produced from the mother's mammary glands-it's a thick, yellow, milky substance secreted during the first few days after giving birth. Colostrum is a rich source of antibodies and is made up primarily of whey protein (75%) and casein. Growth factors and other important protein peptides make up the rest. This "power-packed" nutrient list makes it essential for newborn buffalo infants for proper growth and development [6].

Probiotics contain microbial cells which transit the gastrointestinal tract and which, in doing so, benefit the health of the consumer. *Lactobacilli* are commonly used as probiotic bacteria, in recent years researchers and manufacturers are interested in development and marketing of preparations of living microbial cells [7]. The

activation of the systemic and secretory immune response by *Lactobacilli* requires many complex interactions among the different constituents of the intestinal ecosystem (microflora, epithelial cells and immune cells). Through different mechanisms they send signals to activate immune cells [8, 9].

The large bowel of humans is colonized by a complex microbial community that is often referred to as the intestinal microflora. This community includes possibly hundreds of bacterial species, although it is thought that 30 to 40 species account for 99% of the cells in the community [10]. The collection of bacteria detected in feces reflects the bacteria present in the distal large bowel, so studies of the human intestinal microflora usually involve analyses of the bacterial community in fecal samples [5].

Although these studies have contributed significantly to our understanding of the human intestinal microflora and immune system but the investigators noticed the lacking of a comprehensive understanding of the effect of commercial dairy products on microbial feces composition, tissues, immune response and liver functions in an animal model.

## MATERIALS AND METHODS

**Rats and diets:** Colostrum was milked within 6 hours after buffaloeing from 3 buffaloes of commercial farms in Manshiat el Bakaary region, Egypt. Full fat yoghurt (FFY), fermented milk (FM), fermented milk supplemented with probiotics (FMSP) and whole milk (WM) were purchased from local Egyptian markets and analysed for essential nutrients before mixing with the basal diet Ain-93M diet formulated for maintenance of adult rodents [11]. Bacteriological studies were conducted to investigate the microbiological status of all dairy products mixed with diets.

**Experimental animals:** This study was performed on (n = 54) male at 8 wks of age we, the rats were housed and bred as approved by the Animal Ethics of Ophthalmology Institute Research; Egypt. The animals were kept on rodent chow for a week. After this washout period, rats were divided into 6 groups of 9 rats in each group (n = 9). Rats were kept separately in metal cages in a room with controlled temperature (20 to 22°C) and humidity (50 to 55%) and maintained in a cycle of light for 12 h (0600 to 1800 h) and dark for 12 h (1800 to 0600 h). Albino Swiss male rats were weighing between 115 and 135 g at the beginning of the experiment.

Table 1: Basal diet, Ain-93M

Ingredient	Diet (g/kg)
Cornstarch	465.692
Casein	140.000
Dextrinized corn starch	155.000
Sucrose	100.000
*Corn oil	40.000
Fiber	50.000
Mineral mix	35.000
Vitamin mix	10.000
L-cytestine	1.800
Cholin bitartarate	2.500
Tert-butylhydroquinone	0.008

\*Soybean oil was replaced by corn oil [11]

**Experimental design:** All dairy products were mixed in a percentage of 10% to each basal diet used, Table 1. Rats were divided into six groups as follows I: control group, rats of this group fed on basal diet only Table 1. Group II: buffalo colostrums group (BC), rats of this group fed on fresh buffalo colostrums mixed with basal diet. Group III: fermented milk supplemented with probiotics (FMSP) mixed with basal diet group. Group IV: fermented milk (FM) diet group rats were fed on basal diet mixed fermented milk. Group V: full fat yoghurt (FFY) diet group, the rats of this group were fed on basal diet mixed with full fat yoghurt. Group VI: The rats in this group were fed on of whole milk (WM) mixed with basal diet. Animals of the entire group fed for 30 day on the diets prescribed. Rats were allowed to consume their respective diets and water ad libitum, body weight and feed intake were recorded weekly.

**Immuological and biochemical studies:** At the end of the wk 4 of the feeding trial, rats were fasted overnight and killed by carbon dioxide inhalation. Blood samples were collected immediately in sterile tubes from the retro orbital venous plexus and left to stand for 30 min. at room temperature (~20°C) to coagulate before being centrifuged for 20 min at 2,714 g (Sorvall RT7, Newtown, MA). A number of immunological tests had been used after sacrificing rats such as humoral immune response at the levels of immunoglobulin classes such as serum IgA, IgG & IgM by enzyme linked immune sorbent assay (ELISA). Serum Igs were quantitated by Rat Igs ELISA quantitation Kit, Catalog No. E110-100, E110-128 and E110-102 respectively, (Bethyl laboratories Inc., Japan). Solutions and reagents, step by step method and calculation of results were treated as manufacturer recommendations in Rat IgM, IgG and IgA quantitative ELISA protocol. (Code No. 17194 IBL Co.Ltd, Japan).

Table 2: Mean weight (g) changes in rats treated with of different diets

<sup>a</sup> Group of rats	Initial body weight	Final body weight	Weight gain (g)	Weight gain (%)
Control	130.0±4.99	223.0±4.16	93	72
BC	121.0±2.70	184.0±4.20	63	52
FFY	118.0±0.60	191.0±2.30	73	61
FMSP	120.0±1.90	198.0±1.30	78	65
FM	119.0±1.30	196.0±3.26	77	65
WM	119.0±1.69	200.0±2.10	81	68

BC=Buffalo colostrum, FFY=full fat yoghurt, FMSP=fermented milk supplemented with probiotic, FM= fermented milk, WM= whole milk, ± Standard Error of Mean, n=9

Table 3: Effect of different diets on Got and Gpt serum levels

<sup>a</sup> Groups of rats	Got (U/L)	Gpt (U/L)
Control	137±0.60	72±0.17
BC	<sup>a</sup> 88±0.65	<sup>a</sup> 52±0.33
FFY	<sup>a</sup> 153±0.70	<sup>a</sup> 81±0.23
FMSP	<sup>a</sup> 125±0.38	<sup>a</sup> 60±0.52
FM	<sup>b</sup> 130±0.46	<sup>b</sup> 68±0.35
WM	<sup>a</sup> 123±0.50	<sup>b</sup> 76±0.83

Got= Glutamic-Oxaloacetic Transaminase, Gpt= Glutamic-Pyruvic Transaminase, <sup>a</sup> Results are statistically significant from the control groups of rats (P<0.001). <sup>b</sup> Results are statistically significant from the control groups of rats (P<0.005).± Standard Error of Mean, values are the mean of 9 results, n=9

Table 4: Effect of different diets on serum sodium and potassium ion levels

<sup>a</sup> Groups of rats	Na <sup>+</sup> mEq/L	K <sup>+</sup> mEq/L
Control	144±1.23	4.2±3.19
BC	141±2.00	6.4±4.37
FFY	140±0.30	6.5±2.26
FMSP	141±2.50	6.1±2.59
FM	146±1.80	6.3±1.20
WM	142±2.03	6.6±1.56

±Standard error of mean, values are the mean of 9 results, n=9. Results are statistically significant from the control group of rats (P<0.005)

Tumor necrosis factor alpha (TNF $\alpha$ ), was determined by using kit solid phase sandwich ELISA by using 2 kinds of high specific antibodies; tetra methyl benzidine (TMB) is used as coloring agent (chromogen) the strength of coloring is in proportion to the quantities of rat TNF $\alpha$ .

Moreover, the serum samples were analyzed for serum concentrations of Na<sup>+</sup> and K<sup>+</sup>. Glutamic oxaloacetic transaminase (Got) and glutamic pyruvic transaminase (Gpt) were determined by IFCC method 12 [12].

### Media and culture conditions

**Fecal samples preparation, isolation and enumeration of LAB and bifidobacteria:** Fecal samples were collected freshly from animals after defecation, within 1 h, the

samples then homogenized in sterilized saline, diluted into different concentrations and incubated under the aerobic conditions and under microaerobic conditions (for enumeration of lactobacilli. As well as under anaerobic conditions for enumeration of bifidobacteria. Colony-forming units were counted. In detail, ten grams of feces were homogenized with a stomacher (John Morris Scientific Pty. Ltd., Melbourne, Australia in 90ml Rogosa and Sharpe (MRS) broth supplemented with 0.5% L-cysteine as first dilution and then diluted with 0.85% NaCl, 0.1% peptone and 0.01% cysteine; pH 7.0. Serial dilutions were spread onto Rogosa agar for counting of lactobacilli, Rogosa and Sharpe (MRS) supplemented with 0.5% L-cysteine for count of LAB and the growth of bifidobacteria [13] and MRS supplemented with 100 mg/l neomycin sulfate, 15 mg/l nalidixic acid and 3 g/l lithium chloride for the recovery of bifidobacteria [6]. Plates were incubated in BBL anaerobic jar (Becton Dickinson Microbiology Systems, Sparks, MD) provided with disposable BBL gas generating pack (CO<sup>2</sup> system envelopes, Oxoid, Ltd., West Heidelberg, Victoria) at 37°C for 48 h.

**Histology:** Specimen from organs; liver and colon were fixed in 10% neutral buffered formalin. Sections were routinely processed for light microscopy with formalin fixation, embedded in paraffin and stained with H&E according to Kullisaar *et al.* [14]. All sections were coded and analyzed blindly by the pathologist without knowledge of related characteristics or diet. Histological results have been graded by a scale from 0 to 4 according to severity of histological sections. 0 shows no histopathological results, 1 shows light degree of severity, 2 mild, 3 moderate and 4 severe histopathological results.

**Biostatistics studies:** The data analysis was carried out with SPSS Inc. software (version 15.0). One-way ANOVA was used to study a significant difference between means of the dietary groups with a significance level of P<0.05 and P<0.001 for Table 3-5. All data are presented as ±Standard Error of Means; n = 9.

## RESULTS

**Weight of rats:** In Table 2 all rats were generally healthy throughout the feeding experiment period. The starkest observation of this table is the effect of BC diet group compared to all treatments. WM diet group showed the highest percentage of weight gain compare to dairy diet

Table 5: The effect of different diets on immunoglobulin groups and tumor necrosis factor alpha

<sup>a</sup> Groups of rats	IgG (ng/ml)	Increase over control (%)	IgM (ng/ml)	Increase over control (%)	IgA (ng/ml)	Increase over control (%)	TNF $\alpha$ (ng/ml)
Control	1066 $\pm$ 4.26	0.00	181 $\pm$ 3.55	0.00	61 $\pm$ 1.29	0.00	0.28 $\pm$ 4.30
BC	<sup>a</sup> 1336 $\pm$ 3.04	125.30	<sup>a</sup> 207 $\pm$ 4.23	114.40	<sup>a</sup> 77 $\pm$ 3.20	125.80	0.35 $\pm$ 5.20
FFY	<sup>a</sup> 1110 $\pm$ 5.07	104.10	<sup>a</sup> 202 $\pm$ 2.23	111.60	<sup>a</sup> 79 $\pm$ 4.61	129.20	0.36 $\pm$ 4.60
FMSP	<sup>a</sup> 1160 $\pm$ 3.20	108.80	<sup>a</sup> 195 $\pm$ 5.00	107.90	<sup>a</sup> 83 $\pm$ 3.00	135.80	0.41 $\pm$ 3.91
FM	<sup>a</sup> 1134 $\pm$ 4.23	106.38	<sup>a</sup> 206 $\pm$ 2.26	113.90	<sup>a</sup> 65 $\pm$ 5.20	106.00	0.40 $\pm$ 4.70
WM	<sup>a</sup> 1023 $\pm$ 2.29	96.00	<sup>a</sup> 236 $\pm$ 3.23	130.70	<sup>a</sup> 81 $\pm$ 4.00	131.60	0.37 $\pm$ 2.20

BC=Buffalo colostrum, FFY=full fat yoghurt, FMSP=fermented milk supplemented with probiotic, FM= fermented milk, WM= whole milk,  $\pm$  Standard Error of Mean, values are the mean of 9 results, n=9. <sup>a</sup> Results are statistically significant from the control groups of rats (P<0.001)

Table 6: Count of Lactobacillus Bacteria (LAB) and the growth of bifidobacteria in dairy products (CFU/ml) samples using different selective media

Dairy product	MRS-C (LAB and Bifidobacteria)	MRS>NNL (Bifidobacteria)	Rogosa (Lactobacilli)
FFY	20x10 <sup>8</sup>	7x10 <sup>8</sup>	12x10 <sup>7</sup>
FMSP	35x10 <sup>7</sup>	0	15x10 <sup>7</sup>
FM	15x10 <sup>6</sup>	0	10x10 <sup>6</sup>
BC	30x10 <sup>2</sup>	0	30x10 <sup>2</sup>
WM	0	0	0

Table 7: Count of LAB and the growth of bifidobacteria in animal feces samples (CFU/gm) using different selective media

Group of rats	MRS-C (LAB and Bifidobacteria)	MRS>NNL (Bifidobacteria)	Rogosa (Lactobacilli)
FM	75x10 <sup>8</sup>	15x10 <sup>7</sup>	12x10 <sup>8</sup>
BC	65x10 <sup>8</sup>	10x10 <sup>7</sup>	15x10 <sup>8</sup>
FFY	35x10 <sup>8</sup>	5x10 <sup>7</sup>	10x10 <sup>7</sup>
Control	20x10 <sup>8</sup>	2x10 <sup>3</sup>	30x10 <sup>4</sup>
FMSP	70x10 <sup>8</sup>	7x10 <sup>5</sup>	20x10 <sup>7</sup>
WM	45x10 <sup>3</sup>	0	0

group. Surprisingly, control diet group achieved the highest percentage of body weight gain across the experimented diets.

Table 3 shows a significant reduction of Got and Gpt in BC diet group compared to control diets. On the other hand, FFY diet group increased both Got and Gpt compared to control. Other diets specifically FMSP decreased level of hepatic enzymes a part from whole milk which increased Gpt.

Table 4 shows the level of sodium ions in experimented diets; low levels of sodium have been noticed in all groups except FM diet compared to control groups.

In contrast to sodium results, Table 4 and Fig. 2 show the level of potassium ions in experimented diets, potassium levels were increased in all diets compared to control diet group.

Table 5 and Fig. 3 display the percentage of increasing levels of IgG, IgM, IgA, over control and TNF $\alpha$  levels as noticed. The starkest increase of Igs has been noticed in BC, WM and FMSP diet group. The IgG results demonstrated that, BC diet has the greatest increase over control group and represent 125%. While WM diet increased levels of IgM and IgA to 130.7% and 131.6% over control respectively. On the other hand, the level of TNF $\alpha$  indicated to normal changes within normal values.

Table 6 shows the total counting of lactobacillus and bifidobacteria. *Bifidobacteria* was only found in FFY (7x10<sup>8</sup> cfu log10/g) of fresh fecal weight of rats. Lactobacillus was ranged from 0, 30x10<sup>2</sup>, 10x10<sup>6</sup>, 15x10<sup>7</sup> and 12x10<sup>7</sup> for WM, BC, FM, FMSP and FFY products respectively. Surprisingly no bifidobacteria had not observed in fermented milk supplemented with probiotic.

**Microbial populations in dairy products:** Total microaerobes ranged from 0 to 9.92cfu log10/g of fresh fecal weight of rats across all experimented diets Table 7. Fecal samples from all diets showing increase total microaerobes and anaerobes counts compared with the samples from control group. Rats fed on WM diet showed a lower concentration of total aerobes and microaerobes in the feces samples compared with control group. The FM, FMSP and BC diet groups showed a significant increase in microaerobes and anaerobes compared to control group.

## DISCUSSION

The current work was conducted to compare the effect of consumption of different dairy products which usually consumed as a part of Egyptian diets; buffalo colostrum (BC), full fat yoghurt (FFY), fermented milk (FM), fermented milk supplemented with probiotics (FMSP) and whole milk (WM) in rats. Different

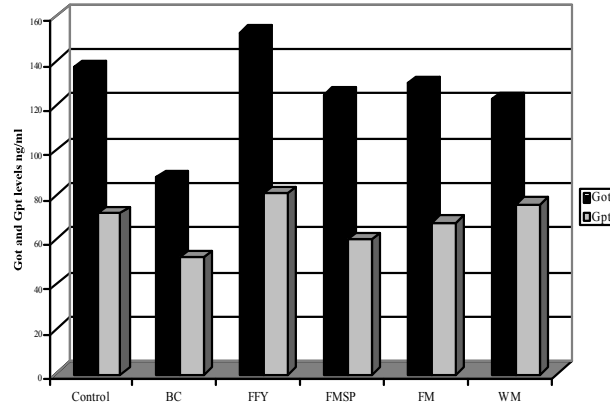


Fig. 1: Serum levels of Got and Gpt distribution among different rat diets  
 BC = Buffalo colostrum, FFY = full fat yoghurt, FMSP = fermented milk supplemented with probiotic, FM = fermented milk, WM= whole milk

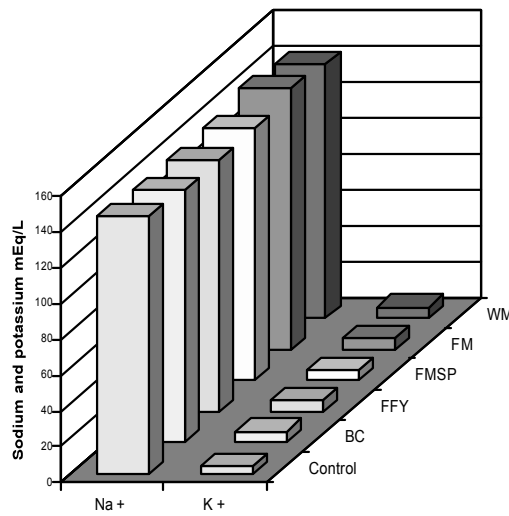


Fig. 2: Effect of different dairy diets on sodium and potassium serum levels

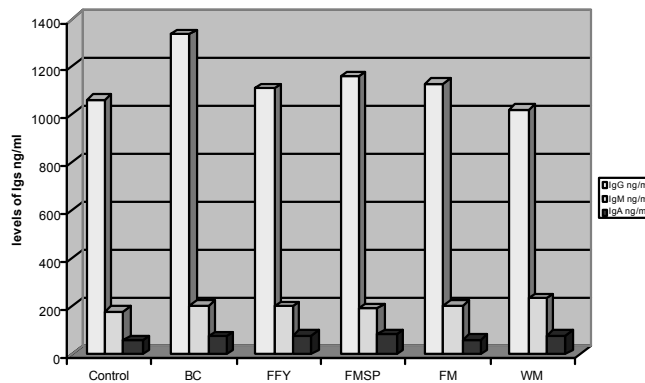


Fig. 4: Effect of different dairy diets on Igs serum levels ng/ml

## Liver histology

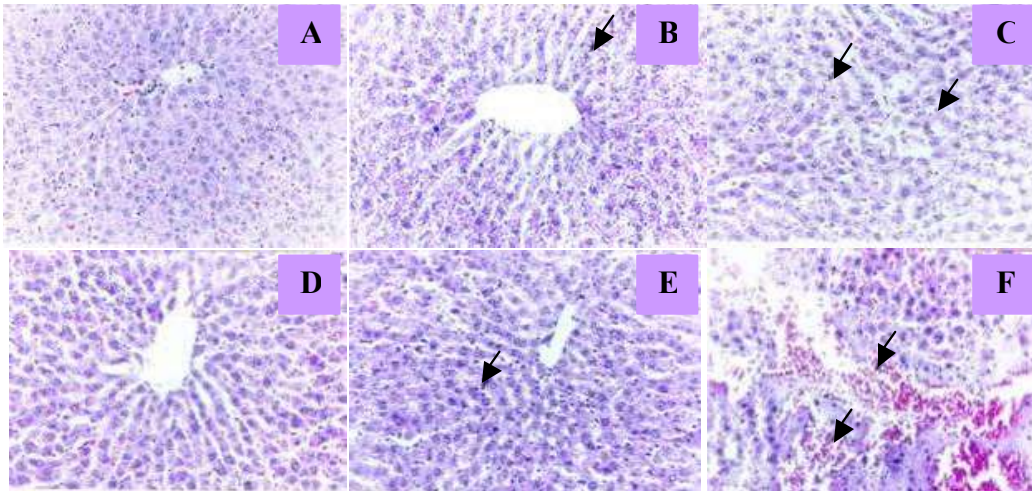


Fig. 5: Liver of rats of control group showing normal histological structure of hepatic lobule (A) (Grade 0) (H and E X 200). Liver of rats fed on FMSP diet showing slight hydropic degeneration of hepatocytes (B) (Grade 1). Liver of rats fed on FFY diet showing vacuolar degeneration of hepatocytes (C) (Grade 2). Liver of WM group showing Kupffer cells activation (D) (grade 3). Liver of rat fed on FM showing focal hepatic hemorrhage dispersed the hepatocytes far away each other cells activation (E) (grade 4). Liver of rat fed on BC diet, showing no histopathological changes (F) (Grade 0)

## Colon histology

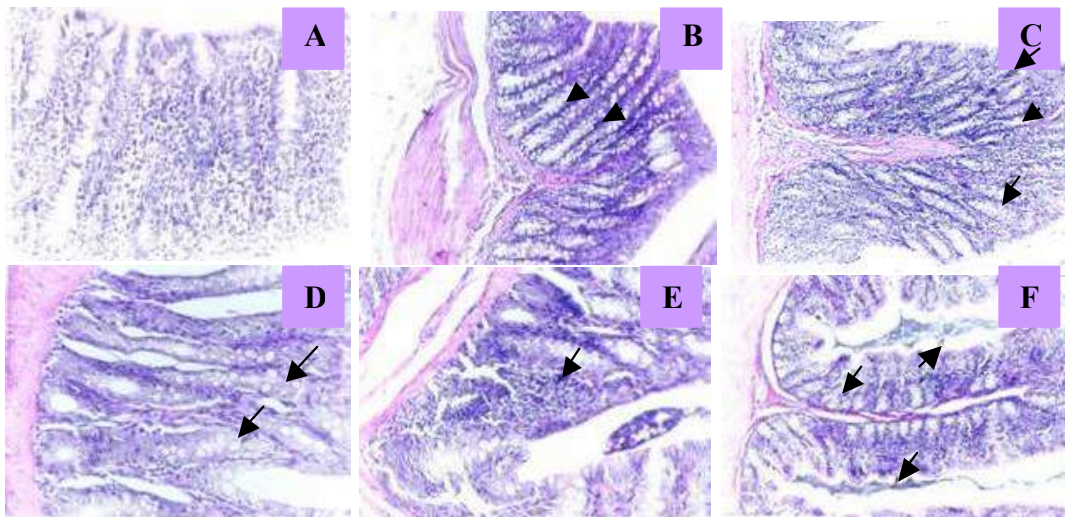


Fig. 6: Colon of rats of control group showing normal mucosa (A) (Grade 0) (H and E X 200). Colon of FMSP diet group showing hyperactivation of mucous secreting glands (B) (Grade 1). Colon of FFY diet group showing hyperactivation of mucous secreting glands (small arrows) associated with leucocytic cells infiltration in lamina propria (large arrows) (C) (Grade 3). Colon of BC diet group showing hyperactivation of mucous secreting glands (D) (Grade 1). Colon of WM diet showing focal mononuclear cells aggregation in lamina propria (arrows) (E) (Grade 2). Colon of FM diet group showing hyperactivation of mucous secreting glands (small arrows) associated with accumulation of basophilic mucous in the lumen (large arrows) submucosal edema arrows (F) (Grade 4)

parameters were carried out; immunological, histological, bacteriological and biochemical that will be discussed.

**Immunity:** The effect of different experimented diets was conducted in rat's serum on the humoral immune response at the levels of immunoglobulin classes IgA, IgG & IgM by Enzyme Linked Immune Sorbent Assay (ELISA). The starkest increase of Igs has been noticed in BC, WM and FMSP diet. The increased levels of immunoglobulin IgG indicate to an activity that influences the natural and adaptive immune systems [15].

The levels of IgG, IgM & IgA over control Table 4 and Fig. 3; IgG results demonstrated that BC diet group has the greatest percentage increase over control and represent 125% that excelled all products compare to control group. The results of David and co-workers [16] studied the safety of New Zealand bovine colostrum on nutritional and physiological evaluation in young rats. They found no difference in colostrum-fed animals and the control group body weight, food consumption, clinical signs, haematology and most parameters of blood chemistry including carbohydrate metabolism, liver function and kidney function. The difference between our results and previous results are using young rats for 90 days and they fed colostrum at 3% and 10% into a normal rat chow but the current study used adult rats and buffalo colostrum diet for only 30 days.

Tumor necrosis factor alpha is a 17.5 kDa, 157 amino acid, protein that is a potent lymphoid factor, which exerts cytotoxic effects on a wide range of tumor cells and other target cells. TNF-alpha has been suggested to play a pro-inflammatory role. It is the primary mediator of immune regulation. The biosynthesis of TNF-alpha is tightly controlled being produced in extremely small quantities in quiescent cells, but is a major secreted factor in activated cells. The level of TNF $\alpha$  indicated to normal changes within normal values. This may be due to the natural effect of milk products.

### Histology

**Histology of liver and colon:** We graded the histopathological results from 0 into grade 4, according to materials and methods. The best results have been noticed in BC diet group that achieved grade 0 and 1 for liver and colon respectively. In contrast, fermented milk achieved grade 4 for both organ tissues compare to control group Fig. 5 and 6. These results may indicate to ensure quality control attributes of the commercial

products before becoming available to the consumers. Colon of rat treated with FM showing hyperactivation of mucous secreting glands associated with accumulation of basophilic mucous in the lumen (large) arrows submucosal edema arrows (Grade 4).

The immune system of the intestine is referred to as GALT (gut-associated-lymphoid tissue). It consists of the Peyer's patches, which are units of lymphoid cells; single lymphocytes scattered in the lamina propria and intraepithelial lymphocytes spread in the intestinal epithelia [17, 18]. It was previously reported that fermented milk had increased the number of beneficial microflora, but the current results specifically histological results indicated the colon condition has not improved markedly specifically FM diet group. Although the time span of the experiment was only 4 weeks it would be suggested that the high concentrations of organic acids, arising from rapid fermentation of prebiotics by probiotics that may inhibit the colonization of acid-sensitive pathogens, could also induce injury to the intestinal mucosa and hence, impair its barrier function [19, 20].

It must also be noted that rats fed on buffalo colostrum diet group were on a scale approaching from normal group. Hierarchically, FMSP diet group came in the third place after BC diet group that may be explained that probiotic action may not necessarily be related to alterations in the composition of the microflora of the large bowel but could have an effect in the small bowel, where the intestinal ecosystem is first exposed to the diet microbes or as we noticed the absence of some probiotic labeled on product.

### Bacteriology

**Dairy products microbial population:** Probiotic bacteria such as *Lactobacillus* are generally regarded as safe (GRAS) for consumption. Until now, reports of harmful effects of these microbes toward a host are rare and their safety has not been questioned. Our results show that *Bifidobacteria* was only found in FFY product, ( $7 \times 10^8$  cfu log<sub>10</sub>/g) compare to other dairy products. There was an increase in the population of lactobacilli and bifidobacteria in fermented milk and FMSP, total microaerobes and anaerobes were ranged from 0,  $30 \times 10^2$ ,  $10 \times 10^6$ ,  $15 \times 10^7$  and  $12 \times 10^7$  for WM, BC, FM, FMSP and FFY respectively. Surprisingly no *bifidobacteria* was found in fermented milk supplemented with probiotic although the labels on the product indicated to that.

Recent research shows that yoghurt bacteria are able to survive passage through the human intestine. The high



production of lactic acid could indicate the possible antimicrobial capability on pathogenic microorganisms. Most feeding dairy studies links between nutrients and microflora composition have been done with supplements such as viable bacteria (probiotics), but in this study we used commercial dairy products available in the Egyptian markets that analysed microbiologically and nutritionally as a step to help the Egyptian dairy industry and governmental surveillance to improve the standards of dairy products.

Our results indicate to the effect FMSP diet contributed to a higher concentration of LAB that may have contributed to the decrease in the population of pathogenic microorganisms that may inhibit the binding of enter pathogenic *E. coli* to intestinal cells [21].

**Feces microbial population:** Fecal samples from all experimented diets showing increase total microaerobes and anaerobes counts compared with the samples from control group. Normally rats supplemented with the WM diet showed the lowest concentration of total aerobes and microaerobes in the feces due to pasteurization process of milk. The FM, FMSP and BC diets showed a significant increase in microaerobes and anaerobes consecutively compared to control group. Results indicate there was an accumulation of probiotic in feces through the 30 days feeding time. It is well recognized that the intestinal microflora influence the digestion and absorption of food, the function of the immune system, peristalsis, production of vitamins such as B-vitamins, vitamin K and influence the turnover of the intestinal epithelial cells.

**Biochemical analysis:** Results from this study also showed a significant decrease of Got and Gpt in BC diet group compared to control diets. On the other hand, FFY diet increased both Got and Gpt compared to control. The immunomodulating capacity *in vivo* of the products derived from this study indicates to the effectiveness of these products to promote health in some diseases such as hepatitis C virus (HCV) that may be suggested after extending the study on subjects. BC diet results indicated that product is safe [16] and affordable.

Many studies link higher intakes of milk and milk products with lower blood pressure and reduced risk of hypertension. Table 3 shows the level of sodium ions in experimented diets that shows low levels of sodium has been noticed in all groups except FM diet compared to control groups. Potassium was increased in all experimented diets compared to control diet. A number of animal studies, epidemiological investigations and clinical trials support that dietary potassium can reduce blood

pressure [22, 23]. It has been suggested that high intakes of dietary potassium may protect against the development of hypertension and improve blood pressure control in those who have high blood pressure [24].

Additional health benefits to dairy foods, a multicentre study of more than 450 adults found that a low fat diet rich in dairy foods (roughly three servings a day), fruits and vegetables (known as the DASH diet) significantly reduced blood pressure within two weeks. A recent reanalysis of this study found that the DASH diet significantly lowers blood pressure in almost subgroups of adults studied (e.g. men and women, older and younger adults, obese and lean individuals, African Americans, sedentary and active individuals [25].

A recent meta-analysis of 32 randomized, controlled trials found that potassium supplementation reduces both systolic and diastolic blood pressure, particularly among hypertensive individuals and people who consume high levels of sodium [26]. The investigators conclude that increasing potassium intake may prove beneficial to prevention and treatment of hypertension, especially among individuals who experience difficulty in lowering their sodium intake. The Nurses Health Study of 300 women with normal blood pressure and habitually low intakes of calcium, potassium and magnesium, linked supplemental intakes of potassium, but not calcium or magnesium, with lower blood pressure [16].

A recent study reports that increasing potassium intake can suppress salt sensitivity in African-American adults [27]. Researchers have proposed several mechanisms for the blood pressure-lowering effects of potassium. These mechanisms include increased urinary sodium excretion and reduced urinary excretion of calcium and magnesium.

In this study, the results are in consistent with the biochemistry, bacteriology, histological and immunological studies. Our observations permit reappraisal of the buffalo colostrum, probiotics, fermented milk and other dairy products. Our results in relevant to nutritional and functional changes on different parameters studied that would challenge the claim of the products equivalency. Such differences should be reflected in naming of these products clearly with clear definition to the bioactive ingredients in the product to avoid consumer confusion.

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### CONCLUSIONS

The main aim of this study was to compare the effects of consumption of different dairy products in rats. Immunological, histological, bacteriological and biochemical parameters were carried out. The BC diet group demonstrated an improvement of parameters proposed. BC diet may be suggested to use in controlling weight programmes, improve liver functions however clear labeling is needed, as well as the insurance of the quality of dairy products. Surprisingly no *bifidobacteria* was found in fermented milk supplemented with probiotic although the labels on the product indicated the presence of it. All the experimented dairy diets result in low sodium and increase potassium levels in serum apart from control group.

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