BIOLOGICAL ATTRIBUTES OF BIO-YOGHURT *VERSUS* THE CONVENTIONAL ONE FED IN SPRAY DRIED FORM

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ABSTRACT

The aim of this study was to evaluate the biological attributes of yoghurt cultured *via* different bacterial starters and fed in the spray dried form.

Cow's milk was converted whether into conventional yoghurt using yoghurt starter culture (YSC) containing of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* or into bio-yoghurt using ABT-2 culture containing of *Lb. acidophilus, Bifidobacterium* sp. and *Str. thermophilus.* At the end of incubation period, yoghurts were spray dried at 160 °C. Milk powder sample was corresponding to the control.

The obtained results revealed that, conventional yoghurt possessed lower lactose content, on dry basis, and higher consistency coefficient as well as yield stress than those of bio-yoghurt, which suffered also from a late development of acidity as well as acetaldehyde content, and consequently relative delayed drop of pH value *versus* the conventional one. Moreover, *Str. thermophilus* count was higher than that of *Lb. delbrueckii* subsp. *bulgaricus*, in the conventional yoghurt. ABT-2 counts seemed trends similar to those of YSC strains, where the count either of *Str. thermophilus* or *Bifidobacterium* sp. was greater than that of *Lb. acidophilus.* After spray drying, con-siderable reductions, with about a logarithmic round were occurred in all strains, but stilled conforming the figures established a minimum of 10^7/g for the starter cultures of fermented milks and a minimum of 10^6/g for specific starter bacteria for which a claim is made for a specific microorganism that has been added as a supplement.

Biologically, a significant improvement in the true digestibility of milk protein was occurred by fermentation especially *via* ABT-2. Conversely, the biological value of protein was higher when rats fed the unfermented products (milk powder) followed by bio-yoghurt and the conventional yoghurt came in the last order. The net protein utilization figures explained that, although the fermentation lowered this criterion, the performance of ABT-2 caused significant amelioration *versus* that done when YSC was used. However, the growth rate of rats was enhanced and the counts of white as well as red blood cells increased by milk fermentation especially with ABT-2 *versus* YSC, although both of hemoglobin and hematocrit % were not influenced. Rats feeding on fermented milk, regardless the kind of bacterial culture were associated with significant increment in the total as well as albumin protein of blood plasma. However, the globulin level was not influenced. The ratio between them was higher in the blood plasma of rats fed on fermented milk. Rats feeding on unfermented milk led significantly to absorb mineral of Ca, P, K, Mg, Zn, Fe and Cu with levels higher than occurred in rats fed on yoghurt, espe-

cially the conventional one. Feeding rats on milk and rather fermented milk products resulted in a considerable increment in lactic acid bacteria and bifidobacterial counts in the feces. Conversely, the coliform bacterial counts were reduced in the feces of these groups.

As conclusions, the ingestion of fermented milk such as yoghurt imparts some biological benefits, especially the protein digestibility and modification of the intestinal flora in order to increase the predominance of specific non-pathogenic bacteria. While, bio-yoghurt was further distinguished with better mineral bioavailability, growth rate and blood picture.

INTRODUCTION

Probiotic microorganisms (*Lactobacillus* sp., *Bifidobacterium* sp. and others) have been defined as live microbial food supplements that affect the host animal beneficially by improving its intestinal microbial balance (**Fuller, 1989 and Brown** *et al* **1997**). It has been suggested that probiotics may be of therapeutic or preventative benefit for a number of pathological states, including: gastroenteritis, diarrhea, constipation and hypercholesterolemia (**Goldin & Gorbach, 1992**). Increasing interest has arisen in the foregoing century in relation to the addition of such species to fermented milks, and a great variety of products containing these have been formulated (**Kneifel & Pacher, 1993 and Ouwehand** *et al* **2003**). Whereas, after ingestion, these cultures must overcome biological barriers that include acid in the stomach and bile in the intestine (**Gilliland, 1978; Kanbe, 1992 and Lankaputhra & Shah, 1995**) and implant in the intestinal tract so as to exert their health-promoting effects there (**Kailasapathy & Rybka, 1997 and Klaver** *et al* **1993**). But, to achieve such therapeutic benefits, a sufficient number of viable microorganisms must be present throughout the entire shelf life of the product. In this regard, minimum levels for probiotic bacteria in fermented milks ranging from 10^5-10^6 colony forming unit (cfu) ml^{-1} (**Samona & Robinson, 1994**) have been suggested. Recently the Codex Alimentarius Commission of FAO/WHO (2002) approved an international standard and established a minimum of 10^7/g for the starter cultures of fermented milks and a minimum of 10^6/g for specific starter bacteria for which a claim is made for a specific microorganism that has been added as a supplement.

Moreover, interest has increased in using probiotic mixtures (either multiple strains of the same species/genus or multiple strains of different genera) instead of a single probiotic strain. This is based on the knowledge that health effects of probiotics are strain specific and thus combining many strains with different properties may result in a more effective product (**Timmerman** *et al* **2004**).

Several studies demonstrated the beneficial health promoting effects of these microorganisms, as it reduces plasma cholesterol and increases the antioxidant activity of different tissues (**Beena & Prasad, 1997; Kawase** *et al* **2000 and Zommara, 2002**), enhances the gastrointestinal tract health by preventing colonization of pathogens, amelioration of diarrhea or constipation, and lactose digestion (**Martini** *et al* **1991; Chen** *et al* **2000; Drouault & Corthier, 2001 and Isolauri** *et al* **2002**), stimulates the immune system (**Perdigon** *et al* **1994**), reduces the diet associated risk of carcinogenesis (**Goldin, 1990 and Perdigon** *et al* **1998**), increasing protein digestion (**Ishibashi & Shimamura, 1993**)and modification of the intestinal flora in order to increase the predominance of specific non-pathogenic bacteria may be employed as an alternative to attain prophylactic or therapeutic effects in intestinal infections and inflammatory conditions. Several mechanisms were suggested to explain how probiotics such as lactic acid bacteria, mediate antinfection effects. These mechanisms comprise the interference with the adhesion of pathogens to the mucosal epithelium (**Reid** *et al* **2001**), the production of antimicrobial substances (**Ogawa** *et al* **2001**), the competition for nutrients (**Sullivan & Nord, 2002**) and the stimulation of host immunity (**Perdigon** *et al* **1986 and Perdigon & Pesce de Ruiz Holgado, 2000**). One of the prerequisites for probiotic action includes survival in the gut and adhesion to specific areas of the gastrointestinal tract.

Therefore, the administration of fermented dairy products can confer enhanced resistance against infections by enteric pathogens (**Ogawa** *et al* **2001 and Reid & Burton, 2002**). Moreover, multispecies-probiotic fermented milk products have proved effective in decreasing the urease activity of *Helicobacter pylori* and aiding in its eradication (**Sheu** *et al* **2002 and Wang** *et al* **2004**), and in reducing the nasal colonization with pathogenic bacteria (**Gluck & Gebbers, 2003**). Nevertheless, the health benefits of the ingestion of fermented milk containing even non-viable lactic acid bacteria (LAB) have been extensively

documented (Holcomb &Frank, 1991; Ouwe-hand & Salminen, 1998; Ouwehand *et al* 2002 and Gibson *et al* 2003).

For that in view, the present study was carried out to evaluate the impact of cow's milk fermentation using different LAB starter cultures rather on biological attributes of resultant products.

MATERIAL AND METHODS

Materials

Fresh cow's milk (3.50% fat and 3.27% protein) was obtained from the herd of Higher Institute of Agric. Co-operation, Shoubra El-Kheima. Full cream milk powder (3.0% moisture, 28.2% fat, 25.7% protein and 37.4% carbohydrate) was obtained from local market made in Egypt. Tow commercial lyophilized bacterial cultures were obtained from Chr. Hansen Laboratory, Copenhagen, Denmark. The first one was the conventional yoghurt starter culture (YSC) containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* and the second was thermophilic culture type ABT-2 DVS containing *Lb. acidophilus, Bifidobacterium* sp. and *Str. thermophilus.*

Experimental procedures

1-Preparation of bacterial starter culture

Lyophilized bacterial cultures were separately inoculated in previously autoclaved (121°C/15 min) fresh skimmed milk (9.6% TS) and incubated at 42°C for the YSC or at 37°C for the ABT-2 type. The complete curdling occurred within 8 h. Starter cultures were freshly used.

2-Preparation of fermented milk

Milk was heat treated to 85°C for 5 min followed by temperature adjustment to 42°C. Then, milk was converted into yoghurt according to the protocol proposed by Tamime & Robinson (1999) with adopting the manufacture conditions enacted by EOSQ (2005), where milk was inoculated with 2% of freshly activated YSC or ABT-2, filled into 1 kg polystyrene containers, covered, and incubated at the same temperature degree (42°C) until complete coagulation (through about 3 h.). Thereafter, the containers, except of those belonged to spray drying, were transferred to the refrigerator (5±1°C), where they were kept to the next day for analyses. Three replicates were done for every treatment. While, the lots aimed to rats feeding were prepared to spray drying.

3- Spray drying of fermented milk

Warm fermented milk (at *ca* 40°C) was exposed to dry using a laboratory-scale spray-drier (A/S Niro Atomizer, Copenhagen, Denmark, NO. 1295). Inlet-air was filtered and heated electrically to 160 °C, as recommended by Kim & Bhowmik (1990), after passing through a blower. The pump delivered the feed solutions to a stainless steel atomizer, with a pressure of 3.5 kg/cm^2. Outlet-air temperatures of 50–60 °C were controlled by adjusting the flow rate of the feed solution as recommended by Kim & Bhowmik (1990) and Lian *et al* (2002). Dried powder samples were collected from the base of cyclone and mixed thoroughly with a spatula. The moisture content of the final powders was less than 5% on the average. The samples were stored in tightly sealed sterile dark bottles at 4 °C.

4-Analytical methods

Dry matter (DM), fat, nitrogen, ash contents and titratable acidity (TA) were determined according to AOAC (2007). Lactose content was calculated by the difference. The acetaldehyde (AC) content was estimated as described by Lees & Jago (1969). The pH value was measured using a pH meter (HANNA Instruments, USA)

Rheological properties of fermented milk were measured at 10°C using a rotary viscometer (RHEOTEST, type RV and Pruefgeraetewerk Medingn, Dresden) as described by Toledo (1980). Consistency coefficient was calculated from the descending flow curve using the following equation:

$$Log\, \delta = log\; \kappa + n\; log\; \gamma$$

Where:

δ　=　Shearing stress, γ = shearing gradient or shear rate,

n　=　Flow behaviour index and κ = consistency coefficient or consistency index.

Whilst, the yield value or yield stress was calculated by fitting the shear stress-shear rate data to the Casson equation (Bourne, 1982).

$$\sqrt{\delta} = \sqrt{\delta o} + \eta a \sqrt{\gamma}$$

Where:

δ = Shearing stress, δo = yield stress, ηa = apparent viscosity and γ = shear rate.

Levels of calcium (Ca), phosphorus (P), potassium (K), sodium (Na) and magnesium (Mg) as major minerals; and zinc (Zn), iron (Fe), cupper (Cu) and manganese (Mn) as minor minerals were determined using an Inductive Coupled Plasma (ICP)-plasma technique, Perkin Elmer-Optima 2000 DV as described in **AOAC (2007)**.

The count of *Lb. delbrueckii* subsp. *bulgaricus* was enumerated using MRS agar (Oxoid) medium as described by **Gueimonde *et al* (2003)**. Culture was incubated anaerobically for 2 days at 37°C. Whilst, *Str. thermophilus, Lb. acidophilus* and *Bifidobacterium* sp. were enumerated using ST agar, MRS-salicin agar and MRS agar media, respectively, after the incubation at 37°C for 72 h. as in **Dave & Shah (1996)**. Coliform was counted using violet red bile agar (VRBA) at pH 7.0-7.2, 37°C for 2 days as in **Hitchins *et al* (1992)**. The count was expressed as colony forming units (cfu)/g of product.

Thiamin (B_1) and Riboflavin (B_2) were measured as described by **Bognar (1992)**, using Beckman HPLC consisting of pump model 126, injector and data handling system. Perkin-Elmer fluorescence detector LC240 and C18 column 25 cm x 4.6 mm were used.

Biological assay was carried out using weighing age Albino male rats of 70-75 g caged individually in the metabolic cages; those were employed for rats comprised an upper living area with feeding system and below a device for the collection of urine and feces. The cages were similar in construction to that described by **Schiller (1960)**, although with a modified method. Acid-washed bottle jars with polyethylene stoppers were used for drinking water and collection of feces and urine (**Horszczaruk & Bock, 1963**) and maintained at 20-24°C and 45-55% relatively humidity. Diets and water were provided fresh daily unless otherwise specified.

Three different diets were previously prepared according to the composition of the diet of **Eggum (1973)** for the 3-subjected experimental animal groups. The 3-diets were different not only in their protein sources but also in their nature as well as the all yoghurt samples were high in fat content in

degree to over the recommended dietary allowance (RDA) of the rats. Thus, the calculation of the nutrients due to the composition of different 3-diets of the corresponding 3 animals groups was according to iso-caloric diet (40 Kcal/rat/day), where starch and oil are expensed in the nitrogen free diet. The rats fed 150 mg nitrogen and 10 g DM/rat/day. The amount of DM is adjusted with N-free diet supplied with vitamins and minerals.

To evaluate the effect of experimental samples on the absorption of nutritional minerals in blood plasma of rats, the procedure of **Eggum (1973)** was applied where rats housed individually in metabolic cages distributed on 3-subjected experimental of 5-rats for each. The 3-groups were fed barley for one-day before starting the experimental to be adapted. The experiment was spread over for a period of 9 days. The animals were weighed at the beginning of the experiment and again at the end. During the experimental period urine of rats was collected in 50 ml of 5% H_2SO_4 while their feces in 100 ml H_2SO_4. Nitrogen of urine and feces was estimated. At the end of experiment all rats were exposed to fasting period of 3 h. and accurately weighed according to **Waynforth & Flecknell (1992)**. Rats were anaesthetized with ether, blood samples were collected from plexieye *via* capillary tube, where total protein and albumin of blood plasma were measured by the photometric system according to the biuret and bromocresol green methods in order (**Johnson *et al* 1999**), while globulin was calculated by the difference between them. Complete blood picture was carried out according to the User's Manual of **RDS (1995)** using Micro-Cobas automatic blood cell counter.

The parameters of the protein quality, namely true digestibility (TD) biological value (BV) and net protein utilization (NPU) were determined according to **Eggum (1973)**.

The obtained data were exposed to statistical analysis according to statistical analyses system user's guide (**SPSS, 1998**).

RESULTS AND DISCUSSION

Chemical and biochemical properties

The obtained results indicated that, there are insignificant differences among yoghurt samples in the levels of all components of their gross composition except of that of lactose, on dry basis, which was in bio-yoghurt higher than that in the conventional one. That was because of slowness

of acid production in the former as reflected on the titratable acidity % and consequently, the bio-yoghurt possessed pH value higher than that of the control. Likewise, the acetaldehyde production was significantly slowed dawn by ABT-2 culture *versus* YSC, (Table, 1). Similar observations were found by **Hussein & Aumara (2006)**. Generally, the results trends of gross composition agree with those reported by **Hagagg & Fayed (1988), Fayed *et al* (1996)** and **Husein *et al* (2006)**.

Table 1. Chemical and rheological properties of cow's milk fermented by different bacterial starter cultures.

Property	Bacterial starter cultures	
	YSC	ABT-2
Dry matter (DM) %	12.85[a]	12.93[a]
Fat/DM %	30.26[a]	30.22[a]
Protein/ DM %	28.96[a]	28.91[a]
Lactose*/ DM%	28.69[b]	29.62[a]
Ash/ DM%	6.25[a]	6.22[a]
Acidity** %	0.75[a]	0.65[b]
pH value	4.50[b]	4.75[a]
Acetaldehyde (μmol/100 g)	3.08[a]	2.45[b]
Consistency coefficient (dyne. sec./ cm^2)	14.80[a]	13.64[b]
Yield stress (dyne/cm^2)	40.94[a]	37.21[b]

The means with the same letter within the same row did not significantly differ (P>0.05).
YSC: Bacterial starter containing of *Streptococcus thermophilus* & *Lactobacillus delbrueckii* subsp. *bulgaricus*.
ABT-2: Bacterial starter containing of *Lb. acidophilus, Bifidobacterium* sp. & *Str. thermophilus*.
* Calculated by the difference.   ** Determined as lactic acid.

Rheological properties

Concerning the rheological parameters, the yoghurt cultured with the type of ABT-2 achieved always the lower figures for the consistency coefficient and yield stress *vis a vis* that cultured with YSC (the control). That could be explained with regard to the relatively lower acidity attained in the former **(Table, 1)** i.e., both consistency coefficient and yield stress of yoghurt matrix are positively acid-induced attributes. Similar findings were reported by **Prentice (1992)** and **Fayed *et al* (2006)**.

Bacterial population

Data given in **Table (2)** show that, the *Str. thermophilus* count is always higher than that of *Lb. delbrueckii* subsp. *bulgaricus* in the conventional yoghurt prior to spray drying. **Rasic & Kurmann (1978)** reviewed that, *Str. thermophilus* grows faster at the beginning of lactic acid fermentation, outnumbering *Lb. delbrueckii* subsp. *bulgaricus* by 3 or 4 times often the 1st h. of incubation. They reported also that, the total count of viable yoghurt bacterial ranges between 200 x 10^6 and 1000 x 10^6 cfu / ml. Similar observations were reported by **Youssef *et al* (2007)**. ABT-2 counts seemed trends similar to those of YSC strain, where the count either of *Str. thermophilus* or *Bifidobacterium* sp. was greater than that of *Lb. acidophilus*, **(Table, 2)**.

Table 2. Bacterial profile (log$_{10}$ cfu /g) of cow's milk fermented by different bacterial starter cultures before and after spray drying.

Strain	Bacterial starter cultures			
	YSC		ABT-2	
	Before	After	Before	After
Lactobacillus acidophilus	ND	ND	7.54[b]	6.74[a]
Bifidobacterium sp.	ND	ND	8.00[a]	6.98[b]
Lb. delbrueckii subsp. *bulgaricus*	7.95[a]	7.00[b]	ND	ND
Streptococcus thermophilus	8.48[a]	7.18[b]	8.75[a]	7.05[b]

The means with the same letter within the same row did not significantly differ (P>0.05).
YSC: Bacterial starter containing of *Streptococcus thermophilus* & *Lactobacillus delbrueckii* subsp. *bulgaricus*.
ABT-2: Bacterial starter containing of *Lb. acidophilus, Bifidobacterium* sp. & *Str. thermophilus*.
Before: before drying.   After: after drying.
cfu: colony forming unit.   ND: not detected.

Regarding the bacterial counts of yoghurts in the relation to spray drying, considerable reductions, with about a logarithmic round, were occurred in all strains, but stilled conforming the figures provided by **Codex Alimentarius Commission of FAO/WHO (2002)**, which approved an international standard and established a minimum of 10^7/g for the starter cultures of fermented milks and a minimum of 10^6/g for specific starter

bacteria for which a claim is made for a specific microorganism that has been added as a supplement. Similar findings were reported by many authors. In experiments carried out by **Teixeira et al (1995)** the beginning total viable count of $L.$ *delbrueckii* subsp. *bulgaricus* was 5×10^9/g reduced to $12.3 - 60.2 \times 10^6$ /g after spray drying depending on the enumeration media used. **To & Etzel (1997)** declared that, during spray-drying, lethal thermal injury is the main reason for reduced cell viability. Moreover, **Lian et al (2002)** found that survival of bifidobacteria after spray-drying varied with strains and is highly dependent on the carriers used. Among the test organisms, $B.$ *longum* B6 exhibited the least sensitivity to spray-drying and showed the highest survival of ca. 82.6% after drying in the presence of 10% skim milk, which achieved the least reduction among other carriers. Furthermore, **Kumar & Mishra (2004)** found that *Str. thermophilus* shows less sensitivity in comparison to $L.$ *delbrueckii* subsp. *bulgaricus*, during spray-drying of yoghurt. Nevertheless, **Porubcan & Sellers (1975)** developed a process for spray drying yoghurt and related cultures such as $L.$ *acidophilus* and $L.$ *helveticus*. They reported however that, the resulting culture powder (s) has a concentration and activity equal to that produced by lyophilization. The spray dried culture powders had viable plate counts of approximately one billion cfu/g, and excellent activities with respect to lactic acid production.

Micronutrients situation

The results also confirmed that, neither the levels (on dry basis) of minerals: Ca, P, K, Na and Mg nor those of trace elements: Cu, Fe, Zn and Mn varied among yoghurt samples regardless the bacterial culture used. Similar trends were behaved with regard to vitamins B_1 and B_2 **(Table, 3)**. These results are in coincidence with those found by **Youssef et al (2007)**. It is noteworthy to mention that, due to the milk powder used was industrially fortified with vitamin B_1, ferric pyrophosphate, zinc sulphate and potassium iodide, the corresponding micronutrients of milk powder exhibited higher figures *versus* yoghurt samples, **(Table, 3)**. These differences have been avoided and eliminated prior the biological experiments to neutralize their factors by the further supplementation with vitamins and minerals as described in the materials and methods section and recommended by **Eggum (1973)**.

Table 3. Micronutrients on dry matter (DM) basis of cow's milk fermented by different bacterial starter cultures.

Component	Milk powder*	Bacterial starter cultures	
		YSC	ABT-2
Minerals (g/kg DM)			
Ca	9.588[b]	11.237[a]	11.237[a]
P	7.732[b]	8.693[a]	8.693[a]
K	13.549[a]	11.339[b]	11.339[b]
Na	3.617[a]	3.409[b]	3.409[b]
Mg	0.876[b]	0.973[a]	0.973[a]
Trace elements (mg/k g DM)			
Cu	2.636[b]	2.825[a]	2.825[a]
Fe	10.319[a]	2.646[b]	2.646[b]
Zn	46.392[a]	39.549[b]	39.549[b]
Mn	0.782[b]	0.825[a]	0.825[a]
Vitamin (mg/kg DM)			
B1	4.124[a]	3.860[b]	3.860[b]
B2	14.433[a]	11.977[b]	11.977[b]

The means with the same letter within the same row did not significantly differ (P>0.05).
YSC: Bacterial starter containing of *Streptococcus thermophilus* & *Lactobacillus delbrueckii* subsp. *bulgaricus*.
ABT-2: Bacterial starter containing of *Lb. acidophilus, Bifidobacterium* sp. & *Str. thermophilus*.
* Industrially fortified with vitamin B_1, ferric pyrophosphate, zinc sulphate and potassium iodide.

Nutritional protein quality

Data present in **Table (4)** elucidated that; a significant improvement in the true digestibility (TD) of milk protein was occurred by fermentation, especially when ABT-2 was performed. Similar findings were reported by **Ishibashi & Shimamura (1993)**. Conversely, the biological value (BV) of protein was significantly higher when rats fed the unfermented products (milk powder) followed by bio-yoghurt and the conventional yoghurt came in the last order. That could be contributed to the lactose content **(Table, 1)**. **Renner & Abd El-Salam (1991)** confirmed that, although the TD decreases, the BV increases with increased dietary lactose content. Whereas, the fraction of absorbed nitrogen excreted as urinary urea nitrogen decreased as dietary lactose in-

creased due to that, the lactose delays the absorption of amino acids, thereby making their utilization more efficient.

Therefore, the net protein utilization (NPU), which a yield of multiplication process of TD×BV explaining that, although the fermentation lowered this criterion, the performance of ABT-2 caused significant amelioration it *versus* that done when YSC was used. However, the growth rate of rats was enhanced by milk fermentation especially with ABT-2 *versus* YSC **(Table, 4)**.

Hargrove & Alford (1978) found that rats fed yoghurt gained weight faster than those fed unfermented or acidified milk. Also, **Akalin** *et al* **(1997)** demonstrated that mice which received commercial chow and yoghurt or acidophilus yoghurt gained higher body weight than those fed only commercial chow. Likewise, **Zammara** *et al* **(2006)** reported that, feeding cultured milk products increased significantly rats- body weight compared to control.

Table 4. Nutritional protein quality and growth rate of rats fed on cow's milk fermented by different bacterial starter cultures.

Property	Milk powder	Bacterial starter cultures	
		YSC	ABT-2
True digestibility %	92.22[c]	93.68[b]	94.20[a]
Biological value %	95.68[a]	85.74[c]	89.00[b]
Net protein utilization %	88.24[a]	80.32[c]	83.84[b]
Growth rate (g/day)	1.94[c]	2.10[b]	2.21[a]

- The means with the same letter within the same row did not significantly differ (P>0.05).
YSC: Bacterial starter containing of *Streptococcus thermophilus* & *Lactobacillus delbrueckii* subsp. *bulgaricus*.
ABT-2: Bacterial starter containing of *Lb. acidophilus, Bifidobacterium* sp. & *Str. thermophilus*.

Blood picture

The results displaying in **Table (5)** reveal that, the counts of white as well as red blood cells increased when rats were fed on yoghurt especially cultured with ABT-2, although both of hemoglobin and hematocrit % were influenced neither with the fermentation nor the kind of bacterial culture used.

Table 5. Blood picture of rats fed on cow's milk fermented by different bacterial starter cultures.

Property	Milk powder	Bacterial starter cultures	
		YSC	ABT-2
White blood cell (10^3/mm³)	5.52[c]	5.82[b]	6.80[a]
Red blood cell (10^6/mm³)	6.38[c]	6.58[b]	6.72[a]
Hemoglobin (g/dl)	12.50[a]	12.55[a]	12.42[a]
Hematocrit %	36.80[a]	36.84[a]	36.90[a]

- The means with the same letter within the same row did not significantly differ (P>0.05).
YSC: Bacterial starter containing of *Streptococcus thermophilus* & *Lactobacillus delbrueckii* subsp. *bulgaricus*.
ABT-2: Bacterial starter containing of *Lb. acidophilus, Bifidobacterium* sp. & *Str. thermophilus*.

Blood plasma profile

Concerning the plasma profile of rats blood data given in **Table (6)** show that, rats feeding on fermented milk, regardless the kind of bacterial culture used, was associated with significant increment in the total as well as albumin protein. However, the globulin level was not influenced. Therefore, the ratio between them was higher in the blood plasma of rats fed on fermented milk.

Nevertheless, the mineral composed of blood plasma indicating that the rats feeding on unfermented milk led to absorb levels significantly higher than occurred in the case of feeding on yoghurts, especially the conventional one, although the experimental diets were previously adjustably supplied with required minerals **(Table, 6)**. This phenomenon could be attributed to the liberation of some protein-bound minerals by the acid produced *via* lactose fermentation. **Renner (1983) and Miller** *et al* **(1995)** declared that, minerals bound to protein are in the forms most available and easily utilized by the body than when present in the ionic forms. The relatively increased lactose level possessed of unfermented milk followed by bio-yoghurt *versus* the conventional one **(Table, 1)** may imparts a further reason to explain this phenomenon. Whereas, **Wasserman** *et al* **(1956)** reported early that lactose produced a great response in promoting mineral absorption. Recently,

Fayed *et al* **(2006)** reviewed that the increased intestinal acidity was aiding absorption of minerals, especially calcium. Similar observations were reported by **Youssef** *et al* **(2007).**

Table 6. Blood plasma profile of rats fed on cow's milk fermented by different bacterial starter cultures.

Property	Milk powder	Bacterial starter cultures	
		YSC	ABT-2
Total protein (g/dl)	5.00[b]	5.23[a]	5.25[a]
Albumin (A) (g/dl)	3.14[b]	3.38[a]	3.39[a]
Globulin (G) (g/dl)	1.86[a]	1.85[a]	1.86[a]
A/G ratio	1.72[b]	1.83[a]	1.82[a]
Mineral (ppm)			
Ca	66.20[a]	53.82[c]	59.62[b]
P	188.50[a]	176.85[c]	180.45[b]
K	145.07[a]	120.85[c]	125.52[b]
Mg	16.69[a]	11.63[c]	13.97[b]
Cu	0.917[a]	0.695[c]	0.782[b]
Fe	5.787[a]	3.092[c]	4.359[b]
Zn	1.625[a]	1.125[c]	1.394[b]

- The means with the same letter within the same row did not significantly differ (P>0.05).
YSC: Bacterial starter containing of *Streptococcus thermophilus* & *Lactobacillus delbrueckii* subsp. *bulgaricus*.
ABT-2: Bacterial starter containing of *Lb. acidophilus*, *Bifidobacterium* sp. & *Str. thermophilus*.

Fecal flora

The effect of the cultured milk products on rats' intestinal microflora are reflected on their feces. Feeding rats on milk and especially fermented milk products resulted in a considerable increment in lactic acid bacteria and bifidobacterial counts in the feces **(Table, 7).** On the other hand, the coliform bacterial counts were reduced in the feces of these groups. This effect was more pronounced in bio-yoghurt fed rats compared to conventional-yoghurt fed groups. These results are in agreement with those of **Yuguchi** *et al* **(1992),** who demonstrated that the administration of bifidobacteria to rats increased its number in the intestine. On the other hand, the numbers of staphylococcus and coliform bacterial counts significantly reduced in groups received cultured milks as com-

pared to the control **Ogata** *et al* **(1997)** found that the percentage of bifidobacteria in the fecal flora of healthy adult volunteers was increased by the administration of *B. bifidum*. Also **Amann** *et al* **(1998)** reported that the consumption of bifidobacteria by human would increase colonic bifidobacteria count. Likewise, **Kheader** *et al* **(2000)** found that the use of a mixture of yoghurt and probiotic strains (bifidobacteria) reduced the growth of entrococci and coliform in the intestinal tract. **Chen** *et al* **(2000)** found that ingestion of yoghurt increased the numbers of stool bifidobacteria and suppressed coliform bacterial count in human intestinal. **Sarkar** & **Misra** **(2002)** found that dietetic yoghurt supplemented with *B. bifidum* created favorable conditions for proliferation of beneficial intestinal microorganisms and discouraged the growth of harmful ones. Similar observations were reported by **Zommara** *et al* **(2006).Gibson** & **Wang** **(1994)** explained that antimicrobial inhibitory effect of *Bifidobacterium* was not related to acid production. Their studies showed that eight species of bifidobacteria could variously secrete an antimicrobial substance with a broad spectrum activity. Moreover, **Medici** *et al* **(2005)** suggested that the protection against enteroinvasive *E. coli* infection observed for the fermented milk containing probiotic bacteria may be associated with an enhance of the intestinal mucosa immunity.

Table 7. Some feces flora ($\log_{10}$ cfu /g) of rats fed on cow's milk fermented by different bacterial starter cultures.

Strain	Milk powder		Bacterial starter cultures			
			YSC		ABT-2	
	Before	After	Before	After	Before	After
A	ND	ND	ND	ND	ND	5.10
B	ND	1.65[a]	ND	2.48[b]	ND	5.28[c]
L	ND	3.15[a]	ND	4.52[b]	ND	5.33[c]
T	ND	3.18[a]	ND	4.60[b]	ND	5.24[c]
E.coli	5.08[a]	5.12[a]	5.07[a]	2.76[b]	5.06[a]	2.18[b]

-The means with the same letter within the same row did not significantly differ (P>0.05).
YSC: Bacterial starter containing of *Streptococcus thermophilus* & *Lactobacillus delbrueckii* subsp. *bulgaricus*.
ABT-2: Bacterial starter containing of *Lb. acidophilus*, *Bifidobacterium* sp. & *Str. thermophilus*
A: *Lactobacillus acidophilus*.
B: *Bifidobacterium* sp.
L: *Lactobacillus delbrueckii* subsp. *bulgaricus*.
T: *Streptococcus thermophilus*.
Before: prior the experimental feeding. After: at the end of the experimental feeding.
ND: not detected.   cfu: colony forming unit.

Finally, it could be concluded that, the ingestion of fermented milk such as yoghurt imparts some biological benefits, especially the protein digestibility and modification of the intestinal flora in order to increase the predominance of specific non-pathogenic bacteria. While, bio-yoghurt was further distinguished with better mineral bioavailability, growth rate and blood picture.

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الخواص الحيوية لليوجهورت الحيوى بالمقارنة بالتقليدى المغذى فى الصورة المجففة بالرذاذ

[١٧]

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استـهدف البحـــث تقيـيم الخـواص الحيويــة لليوجهورت المصنع باستخدام بادئات بكتيرية مختلفة والمغذى على الصورة المجففة بـالرذاذ. حيـث تـم تحويل اللبن البقرى سواء إلى يوجهورت باستخدام بـــادئ اليوجهـــورت المكـــون مـــن *Streptococcus* *thermophilus* و *Lactobacillus delbrueckii* subsp. *bulgaricus* أو إلى يوجهورت حيوى باستخدام ABT-2 المحتــوى علـــــى *Lb. acidophilus* و *Bifidobacterium* sp. و *Str. thermophilus* . وبعـد التحضين تم التجفيف بالرذاذ علـــى درجـة حـرارة ١٦٠°م. وكما تم إستخدام عينة من مسحوق اللـبن المجفف كلبن غير متخمر كتجربة مقارنة فى التغذية.

ولقد أكدت النتائج علـــى إحتـــواء اليوجهــورت التقليدى على نسبة لاكتوز / مادة جافة أقل ومعامـل قوام وجهد قص إبتدائى أعلى من اليوجهوت الحيوى الذى عانى أيضا من تــأخر فـى تطـور الحـامض والأسيتالدهيد وبالتالى تأخر نسبى فى الإنخفاض فـى قيمة الـ pH بالمقارنة بالتقليدى. علاوة على ذلك كان عــــدد *Str. thermophilus* أعلـــــى مـــن *Lb. delbrueckii* subsp. *bulgaricus* فـى اليوجهــورت التقليدى، وكذلك إتخذت سلالات البادئ الحيوى سلوكا

مماثلا لما سبق حيث كانت أعداد *Str. thermophilus* و *Bifidobacterium* sp. أعلى مــن *Lb. acidophilus* فى اليوجهورت الحيوى. ولقد حدث بعـد التجفيـف بالرذاذ إنخفاضات واضحـة بمقـدار حـوالى دورة لوغاريتمية فى أعداد جميع السلالات ولكنها ظلت فى مستوى لم يقل عن١٠٧/ جرام للبكتيريا التقليدية و١٠٦/ جرام للبكتيريا المدعم بها المنتج.

من الناحية البيولوجية فقد حدث تحسن معنوى فى معدل الهضم لبروتين اللبن بالتخمير خاصة باستخدام بادئ ABT-2. مع إنه على العكـس كانـت القيمـة الحيوية للبروتين أعلى عند تغذية الفئران على المنتج غير المتخمر (اللبن المجفف) تلى ذلك اليوجهـورت الحيوى بينما جاء اليوجهوت التقليدى فـى الترتيـب الأخير. كما أوضحت قيم صافي الاستفادة بنيتروجين البروتين إنه برغم أن التخمير أدى إلى خفضها إلا أن التخمير بواسـطة ABT-2 قـد حـسنها بالمقارنـة بالمستخدم بها بادئ اليوجهورت التقليدى. كمـا أن معدل النمو قد تحسن وزادت أعـداد كـرات الـدم الحمراء والبيضاء بالتخمير وخاصة باستخدام ABT-2 بالمقارنة ببادئ اليوجهورت التقليدى، بالرغم مـن الهيموجلوبين أو نسبة الهيماتوكريت (النسبة الحجمية

للكرات الدموية الحمراء بالدم) لم تتأثر. كما ظهر أن تغذية الفئران على اللبن المتخمر بغض النظــر عـــن نوع البادئ المستخدم كان مصاحبا بزيادة معنوية فى البروتين الكلى والألبيومين ببلازما الدم. فى حين أن مستوى الجلوبيولين لم يتأثر. وكانــت النســبة بــين البروتين أعلى ببلازما دم الفئران المغذاة على اللــبن المتخمر. كما أدت تغذية الفئران علــى اللــبن غيــر المتخمر إلى إمتصاص عناصر الكالسيوم، الفوسفور، البوتاسيوم، الماغنسيوم، الزنــك، الحديــد والنحـــاس بمستويات أعلى عن تلك التى حدثت بالتغذيــة علــى اليوجهورت وخاصة التقليدى. وقد نتج عن التغذيــة

على اللبن بصفة عامة وبالأحرى منتجــات الألبـــان المتخمرة زيادة كبيرة فى محتوى براز الفئران مـــن أعداد بكتيريا حمض اللاكتيك و البيفيدو وعلى العكس إنخفضت أعداد بكتيريا القولون به.

ومما سبق يمكن الاستنتاج بأن تناول لبن متخمــر مثل اليوجهورت يضفى بعــض الفوائـــد الحيويــة، وخاصة معدل هضم البروتين وتعديل بكتيريا الأمعاء بحيث تزيد وتسود البكتيريا غير الممرضة. بينما تميز اليوجهورت الحيوى بميزة إضافية تتمثل فى تحـــسين معدل إمتصاص العناصر المعدنيـــة ومعـــدل النمـــو وصورة الدم.