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# DEVELOPMENT OF INFANT FORMULAS BASED ON *Lactobacillus heliveticus* OR *Bifidobacterium lactis Bb12* BACTERIA AND BARLEY

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## ABSTRACT

The present work was designed to prepare fermented porridge by (*Lactobacillus heliveticus* or *Bifidobacterium lactis Bb12*) as baby food with high nutritional value from malt flour, rice flour, skim milk powder, honey and carrot with different percents. Prepared diet were chemically, microbiologically biologically and sensory evaluated directly after fermentation and after storage period (21days) at (4-5 °C). The results indicated that the protein, ash, lactic acid, and acetic acid in all treatment increased during storage period, while fiber, carbohydrate, glucose and fructose values were decreased. The pH values varied according to the growth of both strain, *L heliveticus* recorded a higher decreasing effect on pH values (pH 4.12). The viable count of *L heliveticus* reached maximum growth after storage period 7 days in all treatments, then slightly decreased till the end of the storage period, while *Bifidobacterum lactis Bb12* increased during the storage up to 15 days, and then gradually decreased. All samples had highest values of calcium, phosphorus, magnesium, thiamine and riboflavin to meet Recommended Dietary Allowances (R.D.A) requirements. Sensory evaluation showed that the best formula for infants were samples treated with carrot and both strain . The biological study of fermented porridge by rats indicated that rats body weight gain significantly increased compared to the control with no effect on relative liver and spleen weights. Also, in comparison with the control group, rats fed the fermented porridge resulted in a considerable increase in their intestinal fecal content of *L heliveticus* and Bifidobacteria, however the count of staphylococci and coliforms significantly reduced.

## INTRODUCTION

Human milk is the ideal food for ifants during the first 4 – 6 months of age, since it contains all breast – fed infant nutritional requirements(Tojo *et al* .,1995) . In trying to simulate the breast – fed infant's pattern of gut colonization, the addition of lactobacilli and / or bifidobacteria ( also known as probiotics ) to infant formulas has also been described (Vandenplas, 2002). Probiotics and prebiotics are also incorporated together (known as synbiotics) in foods in order to improve the survival and establishment of beneficial bacteria in the host large intestine, such as addition of probiotics (bacteria) with carbohydrates that promote their growth (prebiotics) to normal infant formula or weaning foods (Edwards and Parrett, 2002).

Cereal can be used as sources of nondigestible carbohydrates that besides promoting several beneficial physiological effects, can also selectively stimulate the growth of lactobacilli and bifidobacteria present in the colon and act as prebiotics. Barley contain water-soluble fiber such as B-glucan and arabinoxylan, oligosaccharides, such as galacto- and fructo-oligosaccharides and resistant starch, which have been suggested to fulfil the prebiotic concept (Charalampopoulos *et al*., 2002) .In addition, barley B-glucan has been reported to selectively support the growth of lactobacilli and bifidobacteria in rat experiments (Ryhanen *et al*., 1996) and in in-vitro studies (Jaskari *et al*., 1993).

Fermentation is an effective method of food preservation. The process of fermentation by lactic acid bacteria (LAB) is capable of lowering the pH to below 4 in food products, including barley-based fermenting cereal gruels used as infant foods.This results in growth reduction of pathogenic bacteria such as *B.cereus, E.coli, Salmonella* spp., *Shigella* spp. and *S.aureus.*(Nout *et al.*, 1989; Simango and Rukure, 1992 and Kingamkono *et al.*, 1995).

Cereals are limited in essential amino acids such as threonine, lysine, and tryptophan, thus making their protein quality poorer compared with animals, and milk (Chavan and Kadam,1989). Lactic acid fermentation of barley has been found effectively to reduce the amount of phytic acid, tannins and improve protein availability (Chavan and Kadam, 1989).Increased amounts of riboflavin, thiamine, niacin, and lysine due to the action of LAB in fermented blends of cereals were also reported (Hamad and Fields,1979). On the other hand, Khetarpaul and Chauhan (1990) reported improved minerals availability of cereal fermented with pure cultures of lactobacilli and yeasts.

The present paper aimed to study the effect of using *Bifidobacterium lactis Bb12*, and *Lactobacillus heliveticus* (probiotic) on the quality of fermented barley porridge (as weaning food )during the storage period (21 days) at refrigerator temperature(4-5°C).

## MATERIALS AND METHODS

-Hull-less barley variety Giza 131 was obtained from the Barley Research Dept. Field Crops Research Insti. A.R.C. Giza, Egypt.

-Skim milk powder was imported from Holland,. It contained 36% protein, 51% lactose, 5% fat, 7.3% Ash and 4% moisture.

-Pure fructose produced and backed by Misr. Scientific Company, Egypt.

-Carrot, natural honey, rice powder were obtained from local markets.

-Starter cultures : *Bifidobacterium lactis* ( *Bb 12*) and *Lactobacillic heliveticus* were supplied by Chr. Hansen Laboratories, Copenhagen, Denmark.

**- Germination of barely seeds and getting the flour:**

Barly seeds was cleaned ,washed and soaked overnight. After that it incubated for 48 hour at 30°C to germination. Then it were dried in an oven at 50°C for 36 h. After that rotes were removed. The germinated barley was ground into flour by using mill machine and sieved through a 250 µm screen.

**-Preparation of fermented barley porridge:**

Malted barley flour (140 g) was blended with 500 ml distilled water for 10 min. and cooked at 90°C for 5 min. Then the skim milk powder (120 g) and pure fructose (10 g) were added slowly to the mixture by using blender. The mixture was pasteurized at 90°C for 30 min., and cooled at 37°C. The mixture was inoculated with 2% *Bifidobacterium lactis Bb 12* or *lactobacillus heliveticus* culture under sterilization condition. The initial acidity of porridge was at pH value 6.7 and the fermentation was run to final pH of 4.8. Fermentation pasteurized carrot 10 g or honey 10 g or both (5g+5g) were added to the mixture, which was then stored at refrigerator temp. (5- 7°C) for 21 days.

-Moisture, protein and fiber contents were determined using the methods described by AOAC. (1990).

-Carbohydrate content was calculated as difference between total weight and the sum of moisture, fat, protein, fiber and ash contents.
-Energy value was calculated by the following formula : 3.47 K cal/ g for protion, 8.38 K cal/g for fat and 4.2 K cal / g for total carbohydrate content.
-pH values were measured according to Ling (1963) using digital pH meter, and total solids was determinate by using Digital Refractometer
-Sugars and organic acids were analyzed by high performance liquid chromatography (HPLC) according the method of Black and Bagley, (1978) and Adhikari *et al.*, (2000).
-Total bacterial counts: Plate count (agar medium) determined according to the method of Lee *et al.*, (1973) plates were incubated for 24 hr at 37 °C.
-*L. heliveticus* were counted using MRS agar, while, *Bifidobacterium lactis* determined by MRS agar + 0.05% L-cystein- HCl according to the method of Dinaker&Mistry (1994).
-*Staphylococcus spp.* were counted using Stph 110 media (Difco, 1974) incubated at 37 °C for 48 h
-Total coliform were estimated by plating suitable dilutions on vilot Red Bile Agar medium as recommended by the APHA (1992). Plates were incubated for 25 h at 37 + 1°C.
-Sensory evaluation was carried out by a regular score panel according to Sanni et al., (1998).
-Biological assay: Old male Albino rats was carried out 3-4 weeks (Food Technology Research Institute (ARE) ,Giza, Egypt.) were acclimatized on commercial chow (Protein 8.2%, Fat 2.6%, Ash 8.6%,Carbohydrate 62.2%, Fiber 7.6% and Moisture 10.8%). The animal were arranged in three groups with six rats in each group. A control group was fed on the commercial chow diet and drank tap water. The other groups were fed on the commercial chow diet and drank the following fermented porridge diluted with water by 80:20, (v/v) for six weeks. Diet and drinks were provided *ad libitum* and fresh fermented porridge were supplied twice a day.
-At the end of the experimental period the small intestines of rats were taken off and washed with 20 ml sterilized saline using sterilized syringe in sterilized flask and serial dilutions were done. Fecal samples were collected from rectum in a sterilized Petri dish and 1.0 g of feces was transferred to flask with 99 ml sterilized water. Samples were analyzed immediately using aseptic sterile dilution technique as described by Klaver *et al.*,(1993).

## RESULTS AND DISCUSSION

Initial pH values of fermented porridge with *Bif.lactisBb-12* and *L. heliviticus* ranged between 4.32 to 4.52are as listed in table (1). Little less acidic pH was observed after 21 days. It was clear that, *L. heliviticus* gave the lowest value of pH in all blends compared with *Bif.lactisBb-12*. These results are in agreement with Ghaly et al., (2003) and Zaki, Hala et al., (2004) who used the whey and *L. heliviticus* for producing pH 4.5 and 5.5. Sneath (1986) mentioned that the optimum pH for initial growth of *Bifidobacterium* was 6.5-

7.0.Also, Kabeir *et al.*, (2005) studied the growth of *Bif.longum BB536* in fermented cereal porridge and their survival during storage up to 21 days at (4-5°C). The low pH value as well as presence of high *Bif. longum BB536* count asserted the safety of the fermented cereals.

**Table (1): Changes of pH value of fermented porridge with *Bif.lactis Bb12* and *L. heliviticus* during storage up to 21 days at (4-5°C).**

| Storage period (days) / Treatments | Fermentation by *Bifidobacterium lactis Bb12.* | | | |
|---|---|---|---|---|
| | Zero time | 7 day | 15 day | 21 day |
| $B^1$ | 4.52 | 4.46 | 4.36 | 4.25 |
| $B^1$.H | 4.46 | 4.36 | 4.34 | 4.27 |
| $B^1$C | 4.43 | 4.38 | 4.28 | 4.20 |
| $B^1$.C.H | 4.40 | 4.36 | 4.29 | 4.23 |
| | Fermentation by *L. heliviticus* | | | |
| $B^2$ | 4.42 | 4.33 | 4.21 | 4.18 |
| $B^2$.H | 4.38 | 4.24 | 4.19 | 4.16 |
| $B^2$.C | 4.32 | 4.25 | 4.17 | 4.12 |
| $B^2$.C.H | 4.37 | 4.21 | 4.14 | 4.12 |

B: barley, B.H: barley with honey, B.C barley with carrot, B.C.H: barley with carrot & Honey. 1 : *L. heliviticus*, 2 : *Bif.lactis Bb-12* .

Data in table (2) show sugars acids profile of fermented carbohydrate using starter cultures during fermentation period. It was found that both glucose and fructose concentration in ($B^1$.H & $B^2$.H) treatment were higher than all other treatments. This may be due to the high ratio of inverted sugar in honey than carrot . In general glucose and fructose values decreased with increasing time of storage.

**Table (2) : Changes of glucose & fructose and the production of lactic acid & acetic acid in fermented porridge with *Bifidobacterum lactis Bb12*and *L. heliviticus* during storage up to 21 days at (4-5°C).**

| Storage period / Treatments | Glucose % | | Fructose % | | Lactic acid % | | Acetic acid % | |
|---|---|---|---|---|---|---|---|---|
| | Zero time | 21 days | Zero time | 21 days | Zero time | 21 days | Zero time | 21 days |
| | Fermentation by *Bifidobacterium lactis Bb12* | | | | | | | |
| $B^1$ | 0.19 | Nd | Nd | Nd | 0.12 | 0.13 | 0.04 | 0.09 |
| $B^1$.H | 6.58 | 5.24 | 5.12 | 2.20 | 0.09 | 0.10 | 0.02 | 0.04 |
| $B^1$.C | 2.77 | 1.16 | 3.34 | 1.56 | 0.01 | 0.08 | 0.02 | 0.04 |
| $B^1$.C.H | 3.89 | 3.19 | 2.96 | 1.19 | 0.09 | 0.12 | 0.02 | 0.04 |
| | Fermentation by *L. heliviticus* | | | | | | | |
| $B^2$ | 1.05 | 0.47 | Nd | Nd | 0.09 | 0.18 | 0.17 | 0.23 |
| $B^2$.H | 6.33 | 1.76 | 3.20 | 1.28 | 0.12 | 0.13 | 0.22 | 0.34 |
| $B^2$.C | 1.36 | 0.58 | 1.30 | 0.86 | 0.14 | 0.15 | 0.28 | 0.37 |
| $B^2$.C.H | 2.19 | 0.66 | 1.26 | 0.52 | 0.11 | 0.12 | 0.18 | 0.24 |

B: barley, B.H: barley with honey, B.C barley with carrot, B.C.H: barley with carrot & Honey. 1 : *L. heliviticus*, 2 : *Bif.lactis Bb-12* .

Sneath *et al.*, (1986) and Holt *et al.*, (1994) described the complete fermentation of sucrose to the high ability of *Bifidobacterum Bb12* to ferment sucrose to fructose and glucose which fermented to acetate and lactate. In case of *L.heliveticus* the values of glucose and fructose were higher than that obtained by *Bif.lactis Bb-12*. Treatment ($B^2$.H)& ($B^1$.H) had similar trends and the highest values of glucose and fructose. The primary functional propertes for lactic acid starter bacteria used in making fermented food products are their ability to produce organic acid by the fermentation of sugar.

The production of lactate and acetate in fermented porridge are shown also in table (2). It could be seen that lactic acid content increased during storage up to 21 days in all treatments. Meanwhile, ($B^1$.C) had the highest value of lactic acid and acetic acid percent. These data agreed with (Kabeir *et al.*, 2005) who reported the low pH (< 4.6) of fermented porridge as results of organic acid such as lactic acid and acitic acid was critical for *Bif.longum*.

Data in table (3) shows the changes in chemical contents of fermented blendes at zero time as stored up to 21 days. In case of fermented porridge by *L.heliveticus* the values of moisture, protein, ash, fiber and T.S.S were rather lower but had the same trend as shown in porridge with *Bif.lactis Bb-12*. This may be due to the lower activity of *Bif.lactis Bb-12*. on carbohydrate fermentation. These results agreed with results obtained by (Zaki, Hala 2004).

Ash percentage values, were high in ($B^1$) & ($B^2$) samples at zero time, and after 21 days of storage, this may be due to the high values of ash in plain barley than carrot or honey; according to FAO (1982), while barley grains, malted barley, carrot and honey (on wet weight basis) had 3.9, 1.8, 0.7, & 0.2% ash respectively.

Fiber percentage values were degraded in all samples after 21 days of storage period. This could be resulted from the fermentation activity of the starter cultures. The results were in harmony with those obtained by Lambo *et al.*, (2005) who indicated that insoluble fiber of barley and oat decreased after fermentation by some strains of L.A.B.

Carbohydrate content values decreased in the following $B^{1,2}$.H, $B^{1,2}$.C.H, $B^{1,2}$.C & $B^{1,2}$. The high values of honey treatments may be due to the high ratio of carbohydrate in it. All treatments after 21 days of storage were of lower carbohydrates in comparison with zero time. These data agreed with that obtained by *Basyony et al.*, (2002).

The values of T.S.S in ($B^{1,2}$.C) were lower than ($B^{1,2}$.H & $B^{1,2}$.C.H). These may be due to the culture activity, acid production and degradation of carbohydrate. The same trend had been obtained by *Basyony et al.*, (2002).

Energy in fermented porridge with *Bif.lactis Bb-12* was higher than porridge with *L.heliveticus* .This may be due to differences of carbohydrate content.

The obtained data in table (4) clearly show higher contents of calcium, phosphorus and iron in treatments ($B^1$ & $B^2$) which consisted of plain barley without honey or carrot. The vailable ingredients levels affect markedly the minerals of final products (Flynn and Cashman,1997). While, the treatments which consist of high ratio of carrot ($B.^1C.$ & $B.^2C.$) showed high content of potassium 232and 231 mg/100g porridge respectively.

Consumption of 100g/days of these formulas would be sufficient to meet the daily requirements of phosphors and magnesium,while 180-230 g/days of these porridges would be enough to cover the RDA of calcium and iron. On the other hand, all prepared porridge contain low levels of sodium and potassium comparing with the permitted amounts RDA (1989).

Also, table (4) indicated that the values of minerals in fermented formula using *L.helveticus* were in general following the same trend of *Bif.lactis Bb-12* .

Also, the data in Table (4) indicated that all prepared porridge samples contained low level of vitamin A comparing with the RDA (1989) which recommended the daily vitamin A intake by infant to be 375 RE/day (1 RE = 6 $\mu$g of all trans $\beta$-carotene). On the other hand treatments ($B^1$.C& $B^2$.C) contained relatively highest values of vitamin A (38.9& 26.6 $\mu g^{RE}$). This may be due to the high ratio of vitamin A in carrot. While, it had high values of thiamine and riboflavin. The high amounts of thiamine may be due to using malt flour in preparing each formula but high ratio of riboflavin may be due to using skim milk powder.

All treatments of fermented porridge with *L.heliveticus* had high values of thiamine and riboflavin than that fermented *by Bif.lactis Bb-12*

The production of lactic acid bacteria during fermentation period lead to 50% higher in the concentration of thiamine and riboflavin in comparing with the initial concentrate in these vitamins (Alm, 1982). Moreover (Gurr, 1987) found the increase in the vitamin content in the formulated cereals blend at the end of the fermentation.

Microbiological analysis of different treatments in zero time and after storage up to 21 days at 4-5°C, using *L. heliveticus* and *Bif. lactis Bb12* were determinated .

Data obtained in fig. (1) show the *L. heliveticus*.count in fermented porridge during the storage up to 21 days at 4-5°C. The counts gradually increased during the storage period(7 days). The count of this strain decreased after storage period (21 days).

Also, data shown in fig. (2) indicate the total count of *Bifidobacterium lactis Bb-12* in the porridge. It might be observed from the results that the maximum population count occurred after 15 days of storage at (4-5 °C). On the other hand at the end of storage period (21 days) data showed marked decrease in the population. This trend agreed with that obtained by (Kabeir *et al.*, 2005) and (Kim *et al.*, 2000). It was clear from figures (1 & 2) that the growth of *Bifidobacterium lactis Bb-12* was lower than *L.heliveticus*. These results were in line with that obtained by Zaki, Hala *et al.*, (2004).

Because foods containing probiotic bacteria should contain at least 6 or 7 log cfu live microorganism per gram or per milliliter at the time of consumption, in order to benefit the consumer (Ishibashiand and Shimamura 1993), all samples of treatments were fully considered as probiotic food.

Coliforms were not detected in all treatments either when zero time or during the storage period. Kunene *et al.*, (1999) and Kingamacono *et al.*, (1995) explained the reduction in level of coliform bacteria by the production of organic acid which would reduce the proliferation of gram negative bacteria and bacterial spores found in fermented porridge.

Table (3): Changes of chemical composition in fermented porridge with *Bif.lactis Bb-12* and *L. helivetícus* after storage up to 21 days at (4-5°C) (on wet weight basis).

| Composition % | Treatment of fermented porridge with *Bifibobacterium lactis Bb12* | | | | | | | | Treatment of fermented porridge with *Lactobacillus heliveticus* | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $B^1$ | | $B^1$.H | | $B^1$.C | | $B^1$.C.H | | $B^2$ | | $B^2$.H | | $B^2$.C | | $B^2$.C.H | |
| | 0 time | 21 days | 0 Time | 21 days | 0 Time | 21 days | 0 Time | 21 days | 0 time | 21 days | 0 time | 21 days | 0 time | 21 days | 0 time | 21 days |
| Moisture | 78.37 | 77.79 | 78.12 | 77.76 | 79.88 | 79.85 | 77.14 | 76.92 | 76.86 | 75.36 | 75.88 | 74.69 | 75.55 | 74.64 | 75.79 | 74.80 |
| Protein | 5.70 | 6.80 | 4.35 | 5.45 | 5.00 | 6.25 | 5.20 | 6.10 | 4.90 | 6.20 | 4.10 | 5.14 | 4.51 | 5.85 | 5.10 | 5.50 |
| Ash | 1.20 | 1.30 | 0.83 | 1.16 | 0.95 | 1.27 | 0.91 | 1.21 | 1.15 | 1.30 | 0.70 | 0.80 | 0.90 | 1.10 | 0.80 | 1.08 |
| Fiber | 1.00 | 0.80 | 0.80 | 0.68 | 1.90 | 1.70 | 0.70 | 0.50 | 1.07 | 0.91 | 0.90 | 0.81 | 2.10 | 1.60 | 1.10 | 0.90 |
| Carbohydrate | 13.73 | 13.31 | 15.90 | 15.04 | 12.27 | 10.93 | 15.89 | 15.27 | 16.73 | 16.73 | 18.42 | 18.56 | 16.94 | 15.41 | 17.21 | 17.02 |
| T.S.S | 12.00 | 14.60 | 25.00 | 26.50 | 13.60 | 15.60 | 18.50 | 19.60 | 11.20 | 12.80 | 22.50 | 20.90 | 12.80 | 11.40 | 17.80 | 16.20 |
| Energy (K.cal) | 77.72 | 80.44 | 81.00 | 81.96 | 69.08 | 68.72 | 84.36 | 85.48 | 86.52 | 90.52 | 90.08 | 95.16 | 85.80 | 85.04 | 89.24 | 90.08 |

B: barley – B.H: barley with honey– B.C: barley with carrot –B.C.H: barley with carrot & Honey
1: *Bif.lactis Bb-12* 2: *L.heliveticus*

Table (4) Minerals and vitamins contents of fermented porridge mg/100g porridge (on wet weight basis).

| Treatments | Ca | P | Mg | Fe | Zn | Cu | Na | K | Vitamin A | | Vitamin C (mg/ 100g) | Thiamine (mg/ 100g) | Riboflavin (mg/ 100g) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | β-carotene (μg /100g) | Vitamin A ($\mu g^{RE*}$) | | | |
| **Fermented porridge with *Bifibobacterium lactis Bb12*** | | | | | | | | | | | | | |
| $B^1$ | 223 | 323 | 47 | 3.07 | 1.77 | 0.24 | 195 | 223 | 16.0 | 2.6 | 1.9 | 17.4 | 3.0 |
| $B^1$.H | 198 | 321 | 46 | 2.82 | 1.45 | 0.12 | 222 | 216 | 27.0 | 4.5 | 2.4 | 17.6 | 3.0 |
| $B^1$.C | 178 | 315 | 46 | 2.40 | 1.25 | 0.24 | 221 | 232 | 133.7 | 38.9 | 4.5 | 17.6 | 4.4 |
| $B^1$.C.H | 190 | 320 | 47 | 2.67 | 1.17 | 0.17 | 243 | 225 | 91.9 | 18.9 | 3.3 | 17.9 | 2.0 |
| **Fermented porridge with *Lactobcillus helveticus*** | | | | | | | | | | | | | |
| $B^2$ | 225 | 328 | 48 | 3.13 | 1.72 | 0.27 | 201 | 222 | 19.0 | 1.5 | 1.8 | 23.8 | 3.6 |
| $B^2$.H | 197 | 324 | 45 | 2.74 | 1.39 | 0.10 | 220 | 209 | 25.0 | 2.8 | 4.1 | 23.2 | 3.4 |
| $B^2$.C | 176 | 313 | 45 | 2.35 | 1.29 | 0.26 | 217 | 231 | 159.0 | 26.6 | 6.0 | 24.8 | 4.8 |
| $B^2$.C.H | 188 | 317 | 49 | 2.59 | 1.24 | 0.19 | 244 | 223 | 99.0 | 16.5 | 5.6 | 22.8 | 3.6 |
| R.D.A (mg) Infants (0-5)months) | 400 | 300 | 40 | 6 | 5 | 0.4 | 120** | 500** | 375 ($\mu g^{RE}$) | | 30 (mg) | 0.4 (mg) | 0.5 – 0.8 (mg) |

**B: barley – B.H: barley with honey – B.C: barley with carrot –B.C.H: barley with carrot & Honey. 1: *Bif.lactisBb-12* 2: *Lb.helveticus* RE*: retinol equivalents. R.D.A: Recommended Dietary Allowances (1989).**

** : Minimum requirements.**

Fig (1): Viability of*L.heliveticu* count (log cfu/g) during *s* storage up to 21 days at (4-5°C).




Fig (2): Viability of Bifidobacterium lactis Bb-12 count (log cfu/g) in fermented porridge during storage up to 21 days at (4-5oC)

Sensory evaluation scores of fermented porridge with *L.heliveticus & Bifidobacterium lactisBb12* during storage up to 21 days was presented in Table (5). Results show that the scores gained for coloure, flavousr, consistency, taste, and texture. The consumer acceptability of porridges production from the different blends showed that all the samples rated above average provided that B.C& B.C.H had the highest preference among treatments which fermented with *Bifidobacterium lactisBb12* or *L. heliveticus*

**Table (5): Sensory evaluation of fermented barley with *Bif.lactisBb12* and *Lb.heliveticus* after storage up to 21 days at 4-5°C.**

| Properties \ Treatments | | Treatments of porridge with *Bif.lactisBb12* and *L. heliveticus* | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | $B^1$ | $B^2$ | $B^1$.H | $B^2$.H | $B^1$.C | $B^2$.C | $B^1$.C.H | $B^2$.C.H |
| Colour | 10 | $7.00^C$ | $7.00^C$ | $6.25^C$ | $7.75^C$ | $8.00^B$ | $9.00^B$ | $7.50^A$ | $8.00^A$ |
| Flavor | 10 | $7.25^B$ | $7.00^C$ | $8.50^B$ | $7.50^B$ | $8.50^B$ | $8.50^B$ | $7.50^B$ | $8.25^B$ |
| Consistency | 10 | $6.25^B$ | $7.00^C$ | $7.00^B$ | $6.50^B$ | $8.00^B$ | $8.50^B$ | $7.00^A$ | $8.00^A$ |
| Taste | 10 | $6.00^B$ | $6.75^C$ | $7.50^B$ | $7.75^C$ | $8.75^A$ | $9.00^B$ | $8.50^A$ | $7.50^A$ |
| Texture | 10 | $5.50^C$ | $6.00^C$ | $7.00^B$ | $6.50^B$ | $8.00^B$ | $8.75^B$ | $7.00^A$ | $8.25^A$ |
| Total | 50 | 32.00 | 33.75 | 36.75 | 36.00 | 41.25 | 43.75 | 37.50 | 40.25 |

B: barley – B.H: barley with honey. – B.C: barley with carrot. –B.C.H: barley with carrot & Honey. 1 : *Bif.lactis Bb-12* 2 : *L. heliveticus*

Table (6) results show the growth parameters of rats. Rats fed on the fermented porridge ($B^1$.C & $B^2$.C) exhibited significantly higher final body weight and body weight gain than the control groups. However, rats' relative liver and spleen weights (organ weight / 100g body weight ) were more or less similar among all groups. These results my be due to probiotic bacteria which produces organic acids such as lactic acid and other by-products such as hydrogen peroxide and antibiotics, and breaks down the bile acids, that improve the intestinal flora and create an environment for the efficient utilization of nutrients (Nakazawa and Hosono, 1992).

**Table (6): Growth parameters of rats fed on fermented porridge (BC) by *L. heliveticus* or *Bif.lactis Bb-12* .**

| Diet groups | Initial body weigh (g) | Final body weigh (g) | body weigh gain(g) | Liver % | Spleen % |
|---|---|---|---|---|---|
| Control | 63.2 + 3.9 | 106 + 12.6 | 43.1 + 10.0 | 4.47 + 0.1 | 0.45 + 0.05 |
| $B^1$.C | 74.2 +7.5 | 161 + 9.5 | 87.2 + 10.5 | 4.77 + 0.4 | 0.64 + 0.05 |
| $B^2$.C | 67.4 + 3.3 | 169 + 3.9 | 102 + 3. 9 | 4.38 + 0.3 | 0.44 + 0.05 |

– B.C: barley with carrot. 1 : *Bif.lactis Bb-12* 2 : *L. heliveticus*

The effect of feeding the fermented porridge ($B^1$.C & $B^2$.C) on small intestinal and feces content of *L. heliveticus* , *Bifidobacteria spp.*, staphylococci and coliform population is shown in table (7). In comparison with the control group, rats fed the fermented porridge resulted in a considerable increase in their intestinal content of *L. heliveticus*, *Bifidobacteria spp.*, however, the count of *staphylococci* and coliforms significantly decreased. These results are in agreement with that obtained by

Patel *et.al*,(1992) Several studies showed the increase of (LAB) & bifidobacteria population and reduction of pathogens microorganisms in human and rats intestinal and feces by feeding on fermented products by (LAB) and *Bifidobacteria spp.* ( Silva *et al.*, 1999, Meddah *et al.*, 2001 and Bruno & Shah, 2002).

**Table (7): Effect of fermented porridge (BC) with *L. heliveticus* or *Bif.lactis Bb-12* on small intestinel and feces.**

| Parameters (log cfu/ml) | Control | B.$^{1}$C | B.$^{2}$C |
|---|---|---|---|
| **Small intestinal** | | | |
| *L.heliveticus* | 7.1 + 0.09 | 10.3 + 012 | 10.5 + 0.13 |
| Bifidobacteria | 6.8 + o.14 | 9.1 + 0.22 | 9.4 + 0.11 |
| Coliforms | 4.2 + 0.10 | 2.9 + 0.23 | 2.4 + 0.13 |
| *Staphylococci* | 4.0 +0.20 | 3.4 + 0.19 | 2.9 + 0.20 |
| ***Staphylococc* Feces** | | | |
| *L. heliveticus* | 7.5 + 0.04 | 10.2 + 0.04 | 10.3 + 0.04 |
| Bifidobacteria | 6.6 + 0.07 | 6.6 + 0.12 | 9.7 + 0.07 |
| Coliforms | 4.8 + 0.11 | 3.1 + 0.12 | 3.1 + 0.09 |
| *Staphylococci* | 4.2 + 0.03 | 4.0 + 0.11 | 2.7 + 0.08 |

**B.C: barley with carrot. 1 : *Bif.lactis Bb-12* 2 : *L. heliveticus***

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## تطوير أغذية الأطفال باستخدام الشعير و بكتريا *Lactobacillus heliveticus* or *Bifidobacterum lactis Bb12*

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تم فى هذه الدراسة تحضير أغذية متخمرة باضافة *Lactobacillus heliveticus* (1) or *Bifidobacterum lactis Bb12* (2) للأطفال ذات قيمة غذائية عالية من دقيق الشعير العارى المنبت, و دقيق الأرز, و اللبن الفرز المجفف, و العسل, و الجزر بنسب مختلفة . حيث تم تقييم هذه الوجبات المحضرة كيميائيا, و ميكروبيولوجيا, و حسيا, و بيولوجيا بعد تجهيزها مباشرة و خلال فترة التخزين لمدة ٢١ يوم على درجة حرارة الثلاجة (٤-٥م) .

و قد أوضحت النتائج زيادة كل من البروتين و الرماد و حمض اللاكتيك و حمض الخليك بعد فترة التخزين, بينما أنخفضت قيم كل من الألياف و الكربوهيدرات و الجلكوز و الفركتوز.

أختلفت قيم الأس الهيد روجينى نتيجة لأختلاف نمو السلالتين فقد سجلت السلالة (١) أقل القيم و هى (٤,١٢ ). كما أظهر العد الكلى لبكتريا السلالة (١) أعلى معدل نمو و حتى اليوم السابع فى كل المعاملات ثم بدأ فى الأنخفاض ببطئ حتى نهاية فترة التخزين. بينما كان
أعلى معدل نمو لبكتريا السلالة (٢) عند اليوم ١٥ ثم حدث انخفاض واضح فى العدد حتى نهاية التخزين .

كما أوضحت النتائج أرتفاع محتوى العينات من أملاح الكالسيوم و المغنسيوم و فيتامينى ب١و ب٢ لتصل الى حدود الأحتياجات اليومية حسب التوصيات العالمية. أما عن النتائج البيولوجية فقد أوضحت أن المنتجات المتخمرة أدت الى زيادة معنوية فى وزن جسم الفئران مقارنة بمجموعة المقارنة بدون أى تأثير على الوزن النسبى (الوزن / ١٠٠ جم من وزن الجسم ) للكبد و الطحال . و بالمقارنة مع مجموعة المقارنة وجد أن الفئران التى غذ يت على المنتجات المتخمرة تميزت بزيادة أعداد بكتريا السلالة (١ و٢) فى أمعائها و برازها فى حين حدث أنخفاض كبير فى أعداد بكتريا القولون و البكتريا العنقودية.