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VIABLE COUNT IN INTESTINE OF RATS FED WITH DIFFERENT YOGURTS

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A Prerequisite for any ingested bacteria to exert a beneficial effect is that these bacteria remain viable during gastric transit and significant number pass to the intestine. Attributed therapeutic benefits of yogurt and other fermented products are thought to be due to intestinal colonisation by the dietary adjuncts used. So it will be useful to have an idea about the *in vivo* fate of yogurt cultures and *B. bifidum*. Hence a study was conducted in rats after feeding yogurts to find out the viable count in intestine.

Materials and methods

Lyophilised yogurt cultures, *S. thermophilus* YH-5 and *L. delbrueckii* ssp *bulgaricus* YH-L (NDRI, Karnal) were transferred to sterilised skim milk separately and incubated at 37° C until coagulation. Three similar transfers were done in the same media for maximum activation. *B. bifidum* 2715 (Reading, UK) was activated in sterilised skim milk containing 1 per cent dextrose and 0.1 per cent yeast extract by incubating at 37° C under carbon dioxide tension till coagulation. Three similar transfers were done in the same media for maximum activation. Routine maintenance of all the three cultures were carried out by fortnightly transfer in sterile skim milk. Between transfers, cultures were kept at 4° C.

Yogurts and Bifidus yogurts were prepared using two methods of fortification viz. skim milk powder (A_1 and B_1) and condensed whey (A_2 and B_2) so as to get 16 per cent total solids in the mix. Cottage cheese whey condensed to 8:1 using a vacuum condenser was used for fortification. Yogurt mixes were inoculated with *S. thermophilus* and *L. delbrueckii* ssp *bulgaricus* in the ratio 1:1 and bifidus yogurt mixes with *S. thermophilus*, L. *delbrueckii* ssp *bulgaricus* and *B. bifidum* in the ratio 1:1:10. The products were prepared as per the standard procedures so as to get a viable count of 10^8 cfu/ml.

Albino rats of uniform weight and age were selected and allotted to four groups. Each group was fed with one type product - Group I, II, III and IV with yogurt A_1, A_2, bifidus yogurt B_1 and B_2 respectively. A control group was maintained without giving any products. Experimental diets were given daily morning at the rate of 10 per cent of body weight. Products were added in the diet so as to replace 50 per cent of the dry matter requirement. Rest 50 per cent of the dry matter requirement was met with a basal rat ration. Rats were caged individually and drinking water was made available *ad libitum*.

After 30 days of feeding and an overnight fasting, rats were anaesthetised and sacrificed. The abdomen was surgically opened and intestinal contents were collected after demarcating the segment by tying. One ml of sterile normal saline of pH 7 was initially injected into the segment. After

mixing, the contents were aspirated out using a sterile syringe. Ten fold dilutions of the aspirated contents were prepared in sterile normal saline and this was plated in duplicate in yogurt lactic agar (Matalone and Sandine, 1986) to get the count of *S. thermophilus* and L. *delbrueckii* ssp *bulgaricus*; in Yoshioka agar (Yoshioka, 1971) to get the count of *B. bifidum* and Mc Conkey agar to get coliform count. The plates were incubated at 37° C for 48 hrs under carbondioxide tension to determine the number of viable *S. thermophilus* and L. *delbrueckii* ssp *bulgaricus*. Mc Conkey agar plates were incubated at 37° C for 24 h to get coliform count. Coliform count of the control group was also taken similarly.

Results and discussion

The results have shown that irrespective of the products fed, in all groups, *S. thermophilus* dominated other organisms in the intestine (Table 1). Thermophilus count was more in group fed with yogurt A_2 and B_2 when compared to A_1 and B_1. In the presence of whey proteins, cultures would have multiplied more (Baig and Prasad, 1995). Increased *S. thermophilus* count in whey protein fortified product fed group may be because, more viable organisms would have entered the system. A similar trend was seen in the case of Bulgaricus count also. Count of L. *delbrueckii* ssp *bulgaricus* in A_2 fed group was more when compared to A_1 fed group. Whey protein supplementation may be the reason for this also. These observations are supported by Goodenough and Kleyn, (1976) who concluded that viable cell population increased in direct proportion to that in natural yogurt and that counts remain elevated until 2-3 h after ingestion of yogurt. Increased acid tolerance of *S. thermophilus* when compared L. *delbrueckii* ssp *bulgaricus* is in accordance with the observations made by Beena and Prasad, (1996) while conducting acid tolerance study of these cultures.

Table 1 Viable count in GI tract of rats fed with experimental diets (10^2 cfu/ml)*

	S. thermo	*L. bulg*	*B. bifi*	*Coliform*
Gr.I (A_1)	243.33±8.82	5.33±0.67	-	119.00±5.51
Gr.II(A_2)	383.33±44.10	96.67±26.03	-	53.33±12.02
Gr.III (B_1)	148.67±16.59	67.67±6.33	124.33±13.86	20.33±9.53
Gr.IV (B_2)	226.67±21.86	24.00±1.15	16.33±7.42	6.00±1.16
Control (10^7)	-	-	-	80.00±5.77

Rocchietta (1975) reported that significant number of yogurt organisms could survive passage through GI tract. Findings of Kolars *et al.* (1984) and Savaiano *et al.* (1984) also support this. In this trial also a significant number of thermophilus and bulgaricus remained viable in intestine.

However counts of *B. bifidum* in group IV (those given bifidus yogurt fortified with

whey proteins) was less when compared to group III. One probable reason for this may be that in the presence of whey proteins yogurt cultures would have multiplied more when compared to Bifidobacteria. The study conducted by Baig and Prasad (1995) have found that *L. delbrueckii* ssp *bulgaricus* has got an inhibiting effect on *B. bifidum*. Reduction in count observed in the present study could also be explained in a similar line.

In the control, only coliform count was taken. In the product fed group viable count of *E. coli* reduced from 10^7 cfu/ml (control) to 10^2 cfu/ml suggesting that using dietary adjuncts can reduce coliform count considerably in the intestine.

According to Fernades *et al.* (1987), in the GI tract, antibiosis due to bacteriocins and antibiotic substances is more relevant. Bacteriocins produced by the cultures used in the product preparation might have played a role in inhibiting/reducing the coliform population in intestine. Hentges (1983) have reported that deconjugated bile salts are more detrimental to the growth of bacteria than conjugated bile salts. Beena and Prasad (1995) have confirmed the bile salt deconjugating capacity of the cultures used here. LAB can deconjugate bile salts and thus suppress food borne pathogens (Fernades and Shahani, 1989). This also supports the observation made here.

Even 12 h after ingestion, 10^2 cfu of the LAB, remained viable in the GI tract suggesting that significant number of organisms survived in GI tract. The results appear to confirm earlier observations made by Shah and Jelen (1990). Even after a feeding trial of 30 days, viable colonies of coliforms were evident showing that dietary supplementation of these bacteria does not replace other intestinal bacteria and that it only helps to maintain a proper balance of resident flora (Kurmann, 1988).

Summary

Rats were fed experimental diets consisting of yogurt and bifidus yogurts containing whey proteins in the form of condensed whey. After a feeding trial of 30 days, rats were sacrificed and the intestinal contents were subjected to plate count to confirm whether the organisms entering the body through the products reached the intestine. The trial confirmed the presence of viable lactic acid bacteria in the intestine. Coliform count reduced considerably in the product fed group when compared to control.

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