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***RESEARCH ARTICLE*.....**

Process optimization for propagation of *Lactobacillus acidophilus* NCFM LYO 10D

Mahadevaiah, H. M. Jayaprakasha **and K.B. Suresha**

ABSTRACT..... The present work was under taken to optimize the propagation of *L. acidophilus* in different mediums for blending into the functional weaning food. A strain of probiotics namely *Lactobacillus acidophilus* NCFM LYO 10D was propagated in whey medium using different prebiotics such as honey, carrot and tomato (CT) juice and whey protein hydrolysate (WPH). The study revealed that 1.5 per cent inoculum and incubation for a period of 15 h incubation at 37^0C is optimum for obtaining the maximum effect in decreasing pH of whey medium. The addition of honey has significant effect in decreasing the pH of whey medium upto 2 per cent honey. Supplementation of honey was also found to have significant effect on the viable count of La-N. The extent of increase in viable count was found be significant upto 2 per cent level. The supplementation of CT juice at a level of 4 per cent was found to have significant effect in decreasing pH and highest viable count. WPH addition has significant effect increase in acidity and decrease pH of whey medium upto 1.5 per cent level. The viable count of La-N significantly increased upon supplementation of WPH. It was concluded that best combination for propogation of La-N in whey medium was found to be supplementation of honey upto 2 per cent, CT juice at a level of 4 per cent, WPH addition upto 1.5 per cent in whey medium and adding 1.5 per cent inoculum and incubating for 15 h at 37^0C.

KEY WORDS..... Propagation, *Lactobacillus acidophilus*, NCFM LYO 10D

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INTRODUCTION.....

Weaning is an important period in the life of a baby. When an infant attains 4-6 months of age, breast milk alone is no longer sufficient to meet its nutritional requirements. Acute diarrhoea and gastrointestinal illness are the major cause of morbidity and mortality in infants and young children during weaning all over the world more so in developing countries. Thus, great care need to be bestowed in selecting and introducing foods during weaning to combat death of millions of infants and suffering hundredths of millions of children from various diseases. Over the past few decades, functional foods have become a topic of increasing importance for the food industry. Many consumers are willing to pay for

products that promise beneficial effects (Suresh, 2009). Cereal-based functional products could be prepared by using cereals as substrates for probiotics and imparting functionality (Charalampopoulos *et al.,* 2002). Probiotics are important functional foods and they comprise approximately 65 per cent of the world functional food market. They can protect against infections, alleviate lactose intolerance, reduce blood cholesterol levels and stimulate the immune system (Agrawal, 2005). The associated beneficial health effects of probiotics could be explored for prevention and treatment of various diseases (Alvarez-Olmos and Oberhelman, 2001 and Guarner and Malagelada, 2003). Such research has stimulated interest in dairy products containing beneficial bacteria for the general public, children and high risk groups (FAO/WHO, 2001). Lactic acid bacteria, specifically lactobacilli and bifidobacteria are the principal representatives of probiotics in the functional food industry (Holzafpel and Schillinger, 2002). Administration of probiotics may have therapeutic and/or preventive benefits in the development of sensitization and atopic disease, particularly in infants with atopic dermatitis (Brouwer *et al.,* 2006), traveler's diarrhoea (Mulder, 2004), atopic eczema/dermatitis syndrome (Viljanen *et al.*, 2005). Functional food ingredients such as prebiotics and probiotics could affect a beneficial modification in the composition and activities of gut microflora of infants by increasing positive flora components. The prebiotic approach aims to increase resident bacteria that are considered to be beneficial for human health, e.g. bifidobacteria and lactobacilli (Parracho *et al.,* 2007). The probiotic currently available in the market are based on lactic acid bacteria, lactobacilli, bifidobacteria and streptococci which have been shown to be important components of gastrointestinal microflora and are relatively harmless (Jayaprakasha *et al.,* 2005). Lactobacillus is one of the most commonly recognized species of the genus Lactobacilli. Whey proteins which possibly have too high a molecular weight to be rendered available for direct bacterial uptake but could be enzymatically cleaved, leading to formation of bifidobacterial growth factors. The highest bacterial growth of 9.1 log cfu/ml was obtained when milk was supplemented with 2 per cent WPC (Janer *et al.,* 2004). Study on the growth of the *Lactobacillus acidophilus* strains revealed that most of them attained maximum growth at 24 h of incubation at 37^0C with a maximum viable count of 9.2 $\log_{10}$ cfu/ml (Borpujari *et al.,* 2007). The morbidity and mortality encountered in infants can be counteracted effectively by feeding such functional weaning foods. Keeping in view the benefits rendered by probiotics the present work was under taken to optimize the propagation of *L. acidophilus* in different mediums for blending into the functional weaning food.

RESEARCH METHODS.....

The ingredients used in the investigation namely Ragi, wheat, green gram, sugar, refined sunflower oil, multivitamins, corn starch, sugar and Daber honey were procured from the local market. Whey Protein Concentrate (WPC) (PROCON 3700 WPC 70) was procured from Mahaan Protein Ltd, New Delhi. Freeze dried probiotic cultures *Lactobacillus acidophilus* NCFM LYO 10 D was procured from Danisco (India) Private Ltd, Mumbai. This culture was sub cultured and maintained at the post graduate laboratory of Dairy Technology Department. Rogosa SL agar M130-500G was procured from HiMedia, laboratories Mumbai to enumerate *Lactobacillus acidophilus* NCFM LYO 10D. Good quality carrot procured from the local market was cleaned and properly washed with potable water. Carrot were cut in to small pieces and grinded in wet grinder followed by filtering and squeezing to get juice. The resultant juice was used for blending with the whey medium. Similarly fresh tomatoes procured from local market were washed with tap water. The washed tomatoes were cut in to small pieces and grinded in wet grinder, the juice obtained was filtered to remove fibre material. Resultant juice was used for blending with whey medium for propagating probiotics. The probiotic culture was propagated in whey medium enriched with different prebiotics at various levels in order to obtain higher viable count. Probiotic culture *Lactobacillus acidophilus* NCFM LYO 10D (La-N) was inoculated separately to the sterilized cheese whey medium at 0.5, 1.0, 1.5 and 2.0 per cent levels and incubated at 37^0C for a period of 24 h. The growth of probiotics as measured in terms of pH, acidity and viable counts was monitored at a regular interval of 3 h. Sterilized whey medium was fortified with various prebiotic namely honey, blend of carrot and tomato juice (1:1) and whey protein hydrolysate. The fortified whey medium was inoculated with probiotic culture and incubated at 37^0C for a known period to study the effect of prebiotics. Cheese whey medium was added

with honey at 1, 2 and 3 per cent levels and subjected to sterilization. Sterilized medium was inoculated separately with Bb-N and La-N at their optimum level and incubated for an optimum period at 37^{0}C as per the results obtained in the para 3.2.2.1. The effect of honey on the activity of probiotics was measured in terms of pH, acidity and viable counts to adjudge the optimum level of honey to be added. Cheese whey medium was added with an optimum level of honey and further added with 3, 4 and 5 per cent levels of carrot and tomato juice blend (1:1) and subjected to sterilization. Sterilized medium was inoculated separately with Bb-N and La-N at their optimum concentration and incubated at 37^{0}C. At a regular interval, the growth of probiotics in terms of pH, acidity and viable counts was monitored to select an appropriate level of carrot and tomato juice to be blended. Cheese whey medium was added with an optimum level of honey and a blend of carrot and tomato juice. Whey medium was further added with 0.5, 1.0, 1.5 and 2.0 per cent levels of Whey Protein Hydrolysate (WPH) and subjected to sterilization. Sterilized whey medium was inoculated with Bb-N and La-N separately at their optimum level and incubated at 37^{0}C. At a regular interval, the growth of probiotics was monitored in terms of pH, acidity and viable counts to elicit the effect of whey protein hydrolysate on the growth of probiotics. The stock solution was prepared by dissolving 3.4 g of Potassium dihydrogen phosphate in 100 ml of distilled water and pH was adjusted to 7.0 with 0.5N NaoH. 1.25ml of stock phosphate buffer solution was taken and the volume was made upto 1000ml with distilled water and distributed in 9ml or 99ml quantities in tubes or bottles and sterilized by autoclaving at 121^{0}C/15 min. 75g Rogosa SL agar media was suspended in 1000 ml distilled water and heated to boiling to dissolve the medium completely. Then 1.32 ml glacial acetic acid was added and mixed thoroughly and distributed into culture tubes or flasks and heated to 90-100^0 C for 2 to 3 min followed by cooling to 40^0 C for direct inoculation. The plates were incubated an anaerobic condition at 37^{0}C for 72-96 h in an anaerobic jar adopting candle method to enumerate *Lactobacillus acidophilus* NCFM LYO 10D. The pH of reconstituted samples was measured using digital pH meter (Elico make). Acidity was measured by titrating against 0.1N NaOH using phenolphthalein indicator and expressed in terms of per cent lactic acid as per the method described in IS:SP:18 (Part XI) 1981. The results were analyzed statistically for test of significance by using Statistical Packages for Social Sciences (SPSS) version 8 software programme.

RESEARCH FINDINGS AND ANALYSIS.....

A strain of probiotics namely *Lactobacillus acidophilus* NCFM LYO 10D was propagated in whey medium using different prebiotics for further incorporation into weaning food. In order to optimize the process to have rich harvest of probiotics, Sterilized cheese whey was inoculated with *Lactobacillus acidophilus* NCFM LYO 10 D (La-N) at 0.5, 1.0, 1.5 and 2 per cent levels and incubated upto a period of 24 h. At a regular interval of 3 h, the activity of the organism was monitored in terms of pH, acidity and viable counts. Similarly, whey medium was further supplemented with honey, Carrot and Tomato (CT) juice and Whey Protein Hydrolysate (WPH). The effect of supplementing these growth promoters on growth and development of La-N is presented hereunder. The results pertaining to the effect of level of inoculum of La-N and duration of incubation on pH, acidity and viable count is presented in Table 1.

Cheese whey was supplemented with honey at 1, 2 and 3 per cent level followed by sterilization. The sterilized whey medium was inoculated with *Lactobacillus acidophilus* NCFM LYO 10 D (La-N) at 1.5 per cent and incubated for a period of 18 h. From the study it is evident that 1.5 per cent inoculum and incubation for a period of 15 h is optimum for obtaining the maximum effect in decreasing pH of whey medium. Many of the earlier workers observed better activity of probiotics in terms of decreasing pH even upto 18 h. Many have suggested an incubation of 18 h for the maximum reduction of pH (Bordignon *et al.,* 2004; Borpujari *et al.,* 2007). It is observed from the result that increasing in the level of inoculum of La-N from 0.5 to 1.5 per cent, there was significant decrease in pH at all durations of incubation. However, above 1.5 per cent, the extent of decrease in pH was not significant. The extent of decrease in pH was observed upto 15 h of incubation. Further incubation upto 18 h had no significant effect in decreasing the pH at any level of inoculums. The pH at 1.5 per cent inoculum was found to be 5.19 with respect to La-N after 15 h of incubation at 37^{0}C. From the results it is evident that both level of inoculum and duration of incubation have significant effect in

decreasing the pH of whey medium. The activity of probiotic as measured in terms of acidity depicted that with increasing in the level of inoculum there is significant increase in the acidity attained in the whey medium irrespective of the type of culture and duration of incubations. A significant increase in acidity was observed from 3 h of incubation upto 15 h of incubation at all levels of inoculum. The extent increase in acidity was found to be non-significant after 15 h of incubation for both La-N indicating that 15 h is optimum enough to get the maximum acidity. Further increase in incubation did not increase the acidity which could be probably due to depletion of inherent nutrients present in the whey system. The acidity development followed the similar pattern as that of pH with an inverse relationship. Several earlier workers reported a maximum acidity of around 0.5 per cent lactic acid after 18 h of incubation (Borpujari *et al.,* 2007). The increased acidity, decreased pH were also reflected with respect to viable counts of probiotics. The extent of increase in viable count with the increase in the duration of incubation followed the similar trend as that of acidity and pH. A maximum viable count of La-N was 6.95 log cfu/ml after 15 h of incubation at 1.5 per cent inoculum. Better activity and higher viable counts of *Lactobacillus* as compared to *Bifidobacterium* species has been reported by several earlier workers (Kusuma Rani, 2006; Santhosh, 2008 and Belkaaloul *et al.,* 2010).

Effect of incorporation of honey on pH, acidity and viable count was monitored. The results are presented in Table 2. As observed from the table, it is evident that addition of honey has significant effect in decreasing the pH of whey medium. With the increase in the level of honey from 0 to 3 per cent, there was significant decrease in pH upto 2 per cent honey supplementation, thereafter the extent of decrease was not significant at

Table 1: Effect of level of inoculum of *Lactobacillus acidophilus* NCFM LYO 10 D on pH, acidity and viable counts in whey medium

Level of inoculums (%)	Period of incubation (h)								Mean
	3	6	9	12	15	18	21	24	
	pH								
0.5	5.66	5.60	5.51	5.45	5.39	5.36	5.33	5.30	5.450
1.0	5.63	5.54	5.46	5.40	5.34	5.30	5.27	5.24	5.397
1.5	5.57	5.47	5.36	5.27	5.19	5.15	5.12	5.09	5.277
2.0	5.55	5.42	5.31	5.22	5.15	5.11	5.09	5.06	5.238
		Levels			Period			Levels x period	
F test		*			*			NS	
C.D. (P=0.05)		0.058			0.047			---	
Acidity (% LA)									
0.5	0.26	0.28	0.31	0.33	0.35	0.36	0.37	0.38	0.330
1.0	0.27	0.30	0.33	0.35	0.37	0.38	0.39	0.40	0.348
1.5	0.29	0.32	0.36	0.39	0.41	0.42	0.44	0.45	0.385
2.0	0.30	0.33	0.38	0.41	0.43	0.44	0.45	0.46	0.400
		Levels			Period			Levels x period	
F test		*			*			NS	
C.D. (P=0.05)		0.016			0.013			---	
Viable count (log cfu/ml)									
0.5				5.63	6.40	6.52	6.15	5.71	6.082
1.0				5.70	6.84	6.65	6.39	5.78	6.272
1.5				5.86	6.95	6.85	6.54	5.84	6.408
2.0				5.90	6.92	6.81	6.49	5.81	6.386
		Levels			Period			Levels x period	
F test		*			*			NS	
C.D. (P=0.05)		0.058			0.098			---	

All values are average of three trials NS= Non-significant

all durations of incubations. The pH of whey medium after 15 h of incubation was 5.19 at no supplementation of honey whereas it was 5.15, 5.01 and 4.98 at 1, 2 and 3 per cent level of honey supplementation. The effect of supplementation of honey also had significant effect on increase in the acidity of whey medium. The acidity of whey medium after 15 h of incubation was only 0.41 per cent lactic acid whereas it was 0.43, 0.48 and 0.50 per cent lactic acid at 1, 2 and 3 per cent level of honey supplementation. It is evident from the result that supplementation of honey has significant effect on extent of increase in acidity and decrease in pH of whey medium. Supplementation of honey was also found to have significant effect on the viable count of La-N. The viable count after 15 h of incubation without honey supplementation was only 6.95 log cfu/ml whereas the supplementation of honey led to increase the viable count to 7.25, 7.58 and 7.53 log cfu/ml indicating the positive effect of supplementation of honey on the activity of La-N.

The extent of increase in viable count was found be significant upto 2 per cent level. Cheese whey was supplemented with 2 per cent honey and added with carrot and tomato juice (1:1 blend) at 3, 4 and 5 per cent level and subjected to sterilization. The sterilized whey medium was inoculated with *Lactobacillus acidophilus* NCFM LYO 10 D (La-N) at 1.5 per cent level and incubated for a period of 18 h. Effect of honey on the growth of probiotics has been reported by some of the earlier workers (Bordignon *et al.*, 2004 and Mendoz *et al.*, 2007). The enhanced activity of probiotic could be attributed to the fructose present in the honey which acts as a prebiotic for the growth of these probiotic organisms. The effect of prebiotic especially fructose has demonstrated a triggering effect on the activity of probiotic.

Table 2: Effect of supplementation of honey on the activity of *Lactobacillus acidophilus* NCFM LYO 10 D in whey medium

Level of honey enrichment (%)	Period of incubation (h)						Mean
	3	6	9	12	15	18	
	pH						
0	5.57	5.47	5.36	5.27	5.19	5.15	5.335
1	5.51	5.42	5.30	5.21	5.15	5.12	5.285
2	5.43	5.28	5.16	5.07	5.01	4.98	5.155
3	5.38	5.25	5.14	5.02	4.98	4.95	5.112
	Levels		Period		Levels x period		
F test	*		*		NS		
C.D. (P=0.05)	0.053		0.042		--		
Acidity (% LA)							
0	0.29	0.32	0.36	0.39	0.41	0.42	0.366
1	0.31	0.34	0.38	0.41	0.43	0.44	0.385
2	0.34	0.39	0.43	0.46	0.48	0.49	0.431
3	0.36	0.40	0.44	0.48	0.50	0.51	0.448
	Levels		Period		Levels x period		
F test	*		*		NS		
C.D. (P=0.05)	0.021		0.016		--		
Viable count (log cfu/ml)							
0				5.86	6.95	6.85	6.553
1				6.17	7.25	6.90	6.773
2				6.74	7.58	6.94	7.086
3				6.79	7.53	6.89	7.070
	Levels		Period		Levels x period		
F test	*		*		*		
C.D. (P=0.05)	0.059		0.710		0.021		

All values are average of three trials NS= Non-significant

The effect of supplementation of carrot and tomato (CT) juice on the activity of the organism in terms of pH, acidity and viable count is presented in Table 3. The supplementation of CT juice was found to have significant effect in decreasing the pH of whey. The pH of whey medium was found to be 5.01, 4.94, 4.82 and 4.78 after 15 h of incubation at 0, 3, 4, and 5 per cent level of supplementation of juice. It is evident from the result that, supplementation of CT juice at a level of 4 per cent was found to have significant effect in decreasing pH. Supplementation at 5 per cent level had no significant effect in further decreasing the pH. Similar trend was also noticed with respect to the pattern of acidity increase. The extent of increase in acidity as a result of supplementation with CT juice was significant upto 4 per cent level, further increase in supplementation had no effect on acidity.

The acidity was found to be 0.48, 0.50, 0.54 and 0.56 per cent lactic acid, respectively at 0, 3, 4 and 5 per cent supplementation level. As could be observed from Table 3, supplementation of CT juice has significant effect on the viable count of La-N. The viable count at 3, 4 and 5 per cent supplementation of CT juice was found to be 8.17, 8.47 and 8.42 log cfu/ml, respectively, whereas it was only 7.58 log cfu/ml when CT juice was not supplemented. However, the extent of increase in viable count was not significant above 4 per cent level.

Cheese whey was supplemented with 2 per cent honey and 4 per cent CT juice and subjected to sterilization. The sterilized medium was added with Whey Protein Hydrolysate (WPH) at 0.5, 1.0, 1.5 and 2.0 per cent level and inoculated with 1.5 per cent of La-N and incubated for a period of 18 h. The effect of supplementation of WPH on the activity of the organism as measured in terms of pH, acidity and viable count is presented in Table 4. From the results it is clear that

Table 3: Effect of supplementation of carrot and tomato juice on the activity of *Lactobacillus acidophilus* NCFM LYO 10 D in whey medium

Level of enrichment (%)	Period of incubation (h)						Mean
	3	6	9	12	15	18	
	pH						
0	5.43	5.28	5.16	5.07	5.01	4.98	5.155
3	5.37	5.22	5.10	5.01	4.94	4.91	5.091
4	5.28	5.09	4.98	4.88	4.82	4.80	4.975
5	5.26	5.07	4.94	4.84	4.78	4.76	4.941
	Levels		Period		Levels x period		
F test	*		*		NS		
C.D. (P=0.05)	0.045		0.051		---		
Acidity (% LA)							
0	0.34	0.39	0.43	0.46	0.48	0.49	0.431
3	0.36	0.41	0.45	0.48	0.50	0.51	0.451
4	0.39	0.45	0.49	0.52	0.54	0.55	0.490
5	0.40	0.46	0.51	0.54	0.56	0.57	0.506
	Levels		Period		Levels x period		
F test	*		*		NS		
C.D. (P=0.05)	0.023		0.013		---		
Viable count (log cfu/ml)							
0				6.74	7.58	6.94	7.086
3				7.23	8.17	7.97	7.790
4				7.62	8.47	8.14	8.076
5				7.65	8.42	8.09	8.053
	Levels		Period		Levels x period		
F test	*		*		*		
C.D. (P=0.05)	0.094		0.715		0.033		

All values are average of three trials NS= Non-significant

WPH addition has significant effect in decreasing pH of whey medium upto 1.5 per cent level, thereafter extent of decrease was not significant. The pH at 0, 0.5, 1.0, 1.5 and 2.0 per cent level of supplementation was observed to 4.82, 4.78, 4.71, 4.58 and 4.56, respectively. The results demonstrated that CT juice has significant effect on the activity of both probiotics. The tomato juice and carrot juice are known to carry certain trace elements and nutrients which probably enhance the activity of probiotic by serving as growth promoters. The effect of CT juice on growth and activity of probiotic has been demonstrated by some of the earlier workers (Yoon *et al.*, 2004 and Tsen Jen-Horng, *et al.*, 2008). Kun *et al.* (2008) reported that, *Bifidobacteria* strains are capable of growing well on pure carrot juice without nutrient supplementation from 10^7cfu/ml to 10^8 cfu/ml after 6 h of incubation. Similarly, the enhanced activity of *Lactobacillus acidophilus* as affected by tomato juice supplementation has been demonstrated by Arora *et al.* (2009) who could able to attain viable counts as high as 8.64 cfu/g. Similar observations were also made by Babu *et al.* (1992) when skimmed milk was supplemented with tomato juice.

It is also evident that supplementation of WPH has significant effect in increasing the acidity of whey upto a level of 1.5 per cent. The acidity was found to be 0.56, 0.58, 0.62 and 0.63 per cent lactic acid at 0.5, 1.0, 1.5 and 2.0 per cent level of supplementation of WPH

Table 4: Effect of supplementation of Whey Protein Hydrolysate (WPH) on the activity of *Lactobacillus acidophilus* NCFM LYO 10 D in whey medium

Level of enrichment (%)	Period of incubation (h)						Mean
	3	6	9	12	15	18	
	pH						
0	5.28	5.09	4.98	4.88	4.82	4.80	4.975
0.5	5.25	5.07	4.92	4.84	4.78	4.74	4.933
1.0	5.19	5.01	4.87	4.76	4.71	4.67	4.868
1.5	5.09	4.92	4.73	4.63	4.58	4.54	4.748
2.0	5.06	4.88	4.70	4.61	4.56	4.52	4.721
	Levels		Period		Levels x period		
F test	*		*		*		
C.D. (P=0.05)	0.038		0.042		0.030		
Acidity (% LA)							
0	0.39	0.45	0.49	0.52	0.54	0.55	0.490
0.5	0.40	0.46	0.51	0.54	0.56	0.57	0.506
1.0	0.42	0.48	0.53	0.56	0.58	0.59	0.526
1.5	0.45	0.51	0.57	0.60	0.62	0.63	0.563
2.0	0.46	0.52	0.58	0.61	0.63	0.64	0.573
	Levels		Period		Levels x period		
F test	*		*		NS		
C.D. (P=0.05)	0.012		0.017		---		
Viable count (log cfu/ml)							
0				7.62	8.47	8.14	8.076
0.5				8.71	8.83	8.56	8.700
1.0				8.83	9.01	8.77	8.870
1.5				8.95	9.09	8.91	8.983
2.0				8.99	9.03	8.97	8.996
	Levels		Period		Levels x period		
F test	*		*		*		
C.D. (P=0.05)	0.071		0.0121		0.037		

All values are average of three trials NS= Non-significant

whereas it was only 0.54 per cent lactic acid at no supplementation of WPH. The viable count of La-N as affected by the supplementation of WPH could also be seen in Table 4.

It is also evident that supplementation of WPH has significant effect in increasing the acidity of whey upto a level of 1.5 per cent. The acidity was found to be 0.56, 0.58, 0.62 and 0.63 per cent lactic acid at 0.5, 1.0, 1.5 and 2.0 per cent level of supplementation of WPH whereas it was only 0.54 per cent lactic acid at no supplementation of WPH. The viable count of La-N as affected by the supplementation of WPH could also be seen in Table 4. The viable count after 15 h of incubation was only 8.47 log cfu/ml when whey medium was not supplemented with WPH, whereas it was 8.83, 9.01, 9.09 and 9.03 log cfu/ml at 0.5, 1.0, 1.5 and 2.0 per cent level of WPH supplementation. The enhanced activity of probiotic could be attributed to the amino acids and peptides which were released as a result of enzymatic hydrolysis of whey proteins. Enzymatic hydrolysis known to release several amino acids and bioactive peptides. Some of these peptides are known to influence the growth of probiotics. It is reported by some of the earlier workers that whey protein hydrolysates enhances the activity of probiotics (Gomes *et al.*,1998). It was observed by Janer *et al*. (2004) that the probiotic counts as high as 9.1 log cfu/ml could be obtained by supplementing milk with 2 per cent WPC. It is confirmed from our result that WPH supplementation enhances the viable count of La-N by one log as compare to without supplementation.

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