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Marine bacteria as source of essential fatty acids : it's application in poultry feed

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It is well established that both linoleic acid and a-linolenic acid are essential fatty acids for entire animal kingdom[1] and are to be consumed through diets. The natural distribution of these two essential fatty acids is not cosmopolitan. The availability of α - linolenic acid is very much restricted and more confined to the marine ecosystem rather than the terrestrial and freshwater ecosystem. This might be the reason for lower level of accumulation of ω3 PUFAs in terrestrial and freshwater animals and higher level of ω3 PUFA in marine animals[2]. It was observed in our laboratory that marine sediments contain about 10% a - linolenic acid in comparison to 5% in brackish water sediments and 0.5% in fresh water sediments (unpublished data).

Marine bacteria are known to produce wide range of compounds, which have potential applications as bioactive compounds, probiotics and nutritional supplements. These organisms are now being screened for the production of polyunsaturated fatty acids as well as specific fatty acids [3,4]. The concept of using microorganisms in feed or enriching the feed with some specific microorganisms in fish is well established in Asian countries. The use of living microbial supplementation in diet as an additional ingredient for enhancing growth of animal has been the thrust area of nutritionist in the recent past. The probiotics have multiple effects on intestinal micro flora and act as health promoting microorganisms [5]. Pujari *et al.*[6] have isolated some bacterial strains having higher lipid content from the sediment collected at a contour depth of 50 and 150 meters from the west coast of India. In the present paper, emphasis is on to find out whether some of these marine bacterial strains can be used as an alternative source of alpha linolenic acid (n-3 fatty acid) and to study their effect on the growth and health of the poultry bird, *Gallus domesticus*, during post hatching development period.

Sediment sample was collected off Mangalore coast at a contour depth of 150 meters during *ORV Sagar Kanya Cruise* during October 2001. The sample was diluted with 0.85% saline and was plated on nutrient agar medium. The predominant bacterial colony was isolated, purified and stored on slants. On the basis of morphological, cultural and biochemical characteristics, the strain was found to belong to genus *Streptococcus*. This selected strain was grown in mineral salt medium (MSM) containing 5% sodium acetate. Cells were then harvested by centrifuging at 10,000 x g for 10min. at 16^{0}C and washed with 0.85% saline. [6]. The harvested bacterial cells were killed by heat treatment and were mixed with the commercial diet to feed the chicks.

Seven days old broiler chicks (Vencobb broiler), *Gallus domesticus* were divided into two groups. Group 1 served as control and the second group was supplemented with streptococcus strain of marine bacteria (0.25g wet bacterial cells per bird per day) along with the commercial feed. The chicks were sacrificed after 30 days of feeding.

Growth of the chicks, in terms of daily instantaneous growth rate (Gw) which was calculated from natural logarithm of weight gain per day and feed conversion ratio (FCR), a ratio of the dry weight of intake feed and the daily weight gain was recorded[7]. Total erythrocytes and leucocytes were counted using Neubauer chamber. The blood hemoglobin was estimated by using Sahli's hemoglobino meter. Tissue protein concentration was recorded for liver, pectoral muscle, and large intestine. The fatty acid profiles of liver, muscle, intestine and serum were analysed with the Gas Chromatogram [8]. The identification of the obtained peeks was done with the prepared standard chromatogram of the known fatty acids under the same programme. Serum lipid profiles including total cholesterol, total triglycerol, HDL cholesterol, LDL-cholesterol, VLDL- cholesterol were also recorded using the diagnostic kits (M/s., Crest Biosystems, Goa, India). The liver function test and the cardiac function test were also performed by measuring the activity of serum Alkaline Phosphatase [EC 3.1.3.1], Lactate Dehydrogenase [EC 1.1.1.27], Glutamate Oxaloacetate Transaminase [EC 2.6.1.1], Glutamate Pyruvate Transaminase [EC 2.6.1.2] as described by Godkar, [9]. All the recorded observation was expressed in the form of arithmetic mean of six

samples and the standard error [10]. The obtained data for each sample group was analyzed with student 't' test.

Linoeic and linolenic fatty acids are very important for animal beings in terms of producing other w3 and w6 series of PUFAs and are involved in temperature adaptation and production of secondary metabolites[11,12].

The concept of using microorganisms in feed or enriching the feed with some specific microorganisms in fish is well established in Asian countries [13,14]. The use of living microbial supplementation in diet as additional ingredient for enhancing the growth of an animal has been thrust area for nutritionist in recent past [15,16]. This probiotic has multiple effects on intestinal microflora and acts as health promoting microorganism [5]. Use of probiotics has become long tradition in animal husbandry [17]. Most frequently used probiotics are associated with lactic acid bacteria [18]. These bacteria often produce bacteriocins and other chemical compounds that might inhibit the growth of other pathogenic bacteria within the animal. Marine bacteria are known to produce wide range of compounds, which have potential application as bioactive compounds, probiotics and nutritional supplements. These microorganisms are now been screened for the production of PUFA as well as specific fatty acids [3,4,6].

The metabolism in bacteria depends upon the carbon sources supplied as growth nutrients which also help in directing the desired accumulation of the metabolites [19]. Induction of oxidation pathways for lipid is found to be regulated with simple carbon sources such as acetate, citrate etc. The bacteria grown in mineral salt medium containing 5% sodium acetate as carbon source showed better growth with 10 times increased lipid concentration, particularly lipid protein ratio and lipid profiles, as compared to the bacteria grown in nutrient broth (Table 1 and 2). Acetyl CoA being common precursor of different lipid molecules, the excess acetate molecules converted into acetyl CoA is then directed towards different biosynthetic routes of lipid molecules [20]. The augmentation of the total fatty acids in the isolates grown in sodium acetate needs to change in the relative fatty acid profiles of the streptococcus. It was interesting to know that a 2 fold augmentation in the conversion efficacy of the alpha linolenic acid (linolenic acid/total C-18 X 100) was noticed in the streptococcus isolate when grown in MSM medium containing 5% sodium acetate (Table 3). Hence an attempt has been made to find out whether this isolate (*Streptococcus*) could be used as a source of alpha linolenic acid in the poultry feed.

Table 1: Percent yield value of total protein and total lipid in bacterial isolate obtained from sediment samples during cruise (Mean ± S. E. of four samples).

Isolate	Protein (mg/100mg of wet wt. of cells)		Lipid (mg/100mg of wet wt. of cells)		Lipid/Protein ratio	
	Nutrient Broth	Sodium Acetate	Nutrient Broth	Sodium Acetate	Nutrient Broth	Sodium Acetate
Strpetococcus sp.	7.16 ± 1.06	5.36 ± 0.74	4.94 ± 0.42	9.57 ± 0.25	0.67	1.78

Table 2 : Comparative table showing the lipid profiles (n mol/mg protein) of bacteria grown on nutrient broth (NB) and sodium acetate (SA) media (mean values of three estimates).

Lipid Profiles	Nutrient Broth	Sodium Acetate
Triglyceride	6.2	54.3
Total Sterol	0.09	5.2
Free Fatty Acids	1.0	10.6
Glycolipid	0.17	5.8
Phospholipid	3.1	27.0
Total Fatty Acids	26.0	233.0

Table 3 : Comparative table showing the fatty acid profiles (relative % composition) of bacteria grown on nutrient broth (NB) and sodium acetate (SA) media (mean values of three estimates).

Fatty acid profiles	Nutrient Broth	Sodium Acetate
C- 12:0	4.85	9.02
C- 14:0	2.53	6.85
C-16:0	7.91	7.20*
C-16:1	2.76	1.27
C-16:2	3.06	2.64*
C- 18:0	7.54	10.98
C- 18:1 (cis)	20.19	20.74*
C- 18:1(trans)	8.04	0.45
C- 18:2 (ω6)	10.93	10.07*
C- 18:2 (ω3)	2.75	1.45
C- 18:3(ω3)	8.03	15.54
C- >18	21.14	13.79
Total C-18	57.48	59.23*
Conversion efficacy of 18:3	13.97	26.24

** The changes are not statistically significant when the same was compared with the isolates grown on nutrient broth media.*

Table 4 : Proximate composition of feeds used in the experiment (Mean values of three estimates and their standard error).

Parameters	Dry matter	Crude fat	Ash	Fiber	Crude protein
Control feed (Commercial)	92.09 ± 1.23	6.50 ± 0.08	10.48 ± 1.76	2.83 ± 0.05	46.28 ± 2.17
Feed + Bacteria	93.25 ± 1.06	10.60 ± 0.11	9.48 ± 1.45	2.74 ± 0.04	46.53 ± 2.16

Table 5 : Relative composition of fatty acid profiles of feed supplemented with different lipid sources used in the experiment (Mean values of three estimates).

Fatty acid	Control feed (Commercial)	Feed+Bacteria
14:0	7.50	7.45
16:0	16.00	11.62
16:1	2.30	1.75
18:0	8.30	19.26
18:1	2.50	11.25
18:2	55.50	32.64
18:3	0.50	7.85
Other fatty acids of C 10-16 series	7.40$	8.18#

- Short chained fatty acids of C –10 and C –12 series
$ - Unidentified fatty acids of C –10 and C –12 series

The observed 30% increase in growth in terms of net weight gain of the bird along with 25% decrease in FCR with supplementation of *Streptococcus* strain of bacteria in diet for a period of 30 days (Table 6) indicates the bacterial role as growth promoting micro-organisms. The increased net weight gain of the bird with bacterial supplementation is reflected in the liver and muscle protein concentration (Figure 2). The increased growth might be due to the increase in crude fat content in the experimental diet supplemented with bacteria (Table 4). These observations once again confirm the involvement of dietary fat to prevent utilization of dietary proteins in energy yielding process and thus is in agreement with the earlier findings of Manju and Dhevendaran [16] reported that single cell protein (SCP) of microbial origin appears to be a 25% - 50% substitute for fish meal for the growth of juvenile prawn. The present result once again confirms the dietary role of a - linolenic acid in growth and lipid metabolism of the bird. Secondly, increase in the relative concentration of eciosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) along with decrease in oleic acid, linoleic acid and arachidonic acid in various tissues of chicks (Table 7) due to bacterial supplementation in diet over a period of 30 days once again confirm the competition of α - linolenic acid with linoleic acid to bind with r5 and r6 desaturases enzyme system for the production of long chain PUFA. The similar observations were made on dietary supplementation of n3 fatty acid rich fish oil in chickens [21-24].

Table 6 : Growth chart of Chick (*Gallus domesticus*) supplemented with bacteria along with the commercial feed for 30 days during post hatching development (Mean values of six samples and their standard error).

Parameters	Control	Bacteria suppl.
Net weight of the birds (g)	1200.00±115.67	1575.70±120.30
Daily instantaneous growth rate (Gw)	0.234	0.244
Feed Conversion ratio (FCR)	1.062	0.797

Table 7 : Fatty acid profiles (relative percent composition) of liver total lipid of chick (*Gallus domesticus*) supplemented with bacteria along with the commercial feed for 30 days during post hatching development (Mean values of 3 sets of samples are presented).

Fatty acid	Control	Bacteria supplemented
Liver		
16:0	29.8a	27.1
16:1	4.20a	6.00
18:0	18.2c	20.50
18:1	15.2a	10.50
18:2 (ω6)	15.3a	12.00
18:3(ω3)	1.20a	3.00
20:4(ω6)	10.20a	7.00
20:5(ω3)	2.40a	6.50
22:6(ω3)	1.40a	4.50
others	2.10	2.90
Muscle		
16:0	28.30a	24.50
16:1	3.60	4.10
18:0	14.30	14.60
18:1	19.30a	14.20
18:2 (ω6)	14.00a	10.50
18:3(ω3)	2.50a	5.00
20:4(ω6)	9.30a	7.00
20:5(ω3)	2.20a	5.80
22:6(ω3)	2.60a	5.50
others	3.90	8.80

contd.

contd ..		
Intestine		
16:0	27.50a	26.50
16:1	4.40a	4.80
18:0	10.20a	11.20
18:1	27.20a	20.70
18:2 (ω6)	16.50a	14.50
18:3(ω3)	2.40a	5.20
20:4(ω6)	7.50c	6.50
20:5(ω3)	1.50c	3.00
22:6(ω3)	1.40c	3.80
others	1.40	3.80
Serum		
16:0	22.67a	20.78
16:1	1.20a	3.50
18:0	23.45a	22.12
18:1	11.35a	8.54
18:2 (ω6)	26.37a	21.00
18:3(ω3)	0.50 a	2.50
20:4(ω6)	10.34a	7.80
20:5(ω3)	1.06 a	5.70
22:6(ω3)	1.30 a	4.80
others	1.76	3.26

a - Changes are significant ($P< 0.01$)

Dietary supplementation of bacteria as a source of α - linolenic acid (Table 5) did not significantly alter the hemoglobin concentration and total erythrocyte count in the blood. However, more than two fold increase was recorded in the total leukocyte count of the chicks (Figure 1). The enhanced leukocyte count in the blood may be correlated with increased immuno protective conditions with supplementation of alpha linolenic acid. Sijben *et al.*[25] reported that the dietary fatty acids of w3 series plays a significant role in the immuno response mechanism of growing layer hen by controlling the actions of the different antigens. Decrease in the concentration of total cholesterol and triglycerol in the serum along with

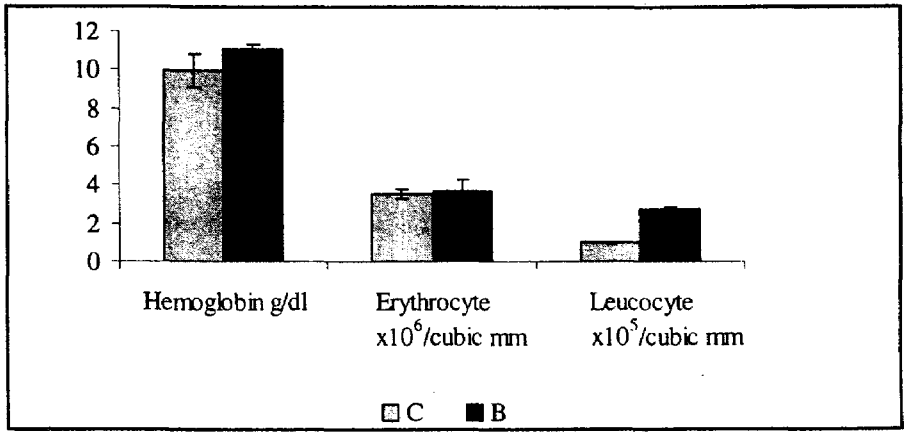

Figure 1: Changes in the hematology of the chick (*Gallus domesticus*)supplemented with bacteria along with the commercial feed for 30 days during post hatching development. C—— Control ; B— Bacteria supplemented.

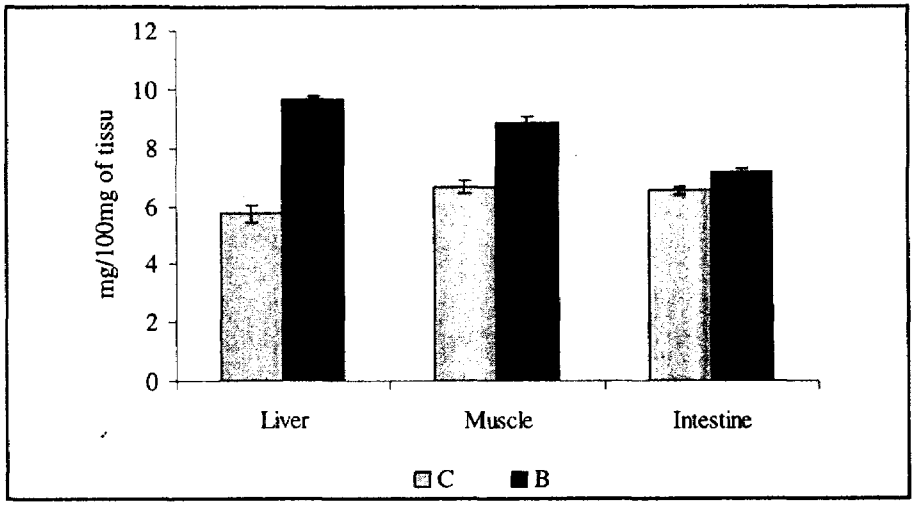

Figure 2: Changes in the tissue protein concentration of the chick (*Gallus domesticus*) supplemented with bacteria along with the commercial feed for 30 days during post hatching development. C— Control ; B— Bacteria supplemented.

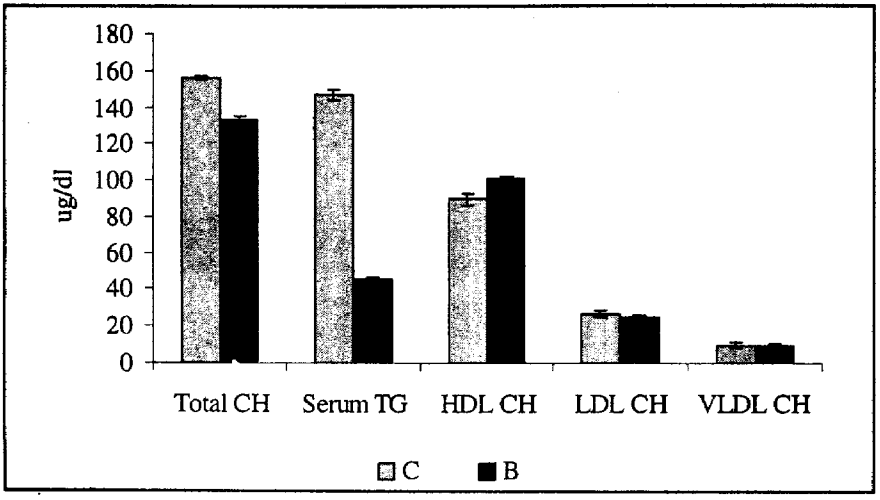

Figure 3: Changes in the serum lipid profiles of the chick (*Gallus domesticus*) supplemented with bacteria along with the commercial feed for 30 days during post hatching development. C—— Control ; B— Bacteria supplemented.

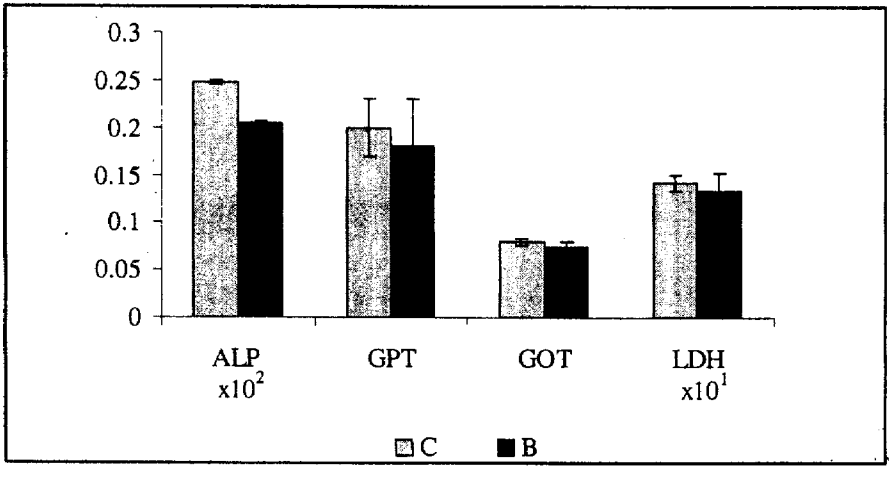

Figure 4: Changes in the activities of some enzymes in serum of the chick (*Gallus domesticus*) supplemented with d bacteria along with the commercial feed for 30 days during post hatching development. C—— Control; B— Bacteria supplemented.

little increase in HDL cholesterol concentration without altering LDL or VLDL cholesterol due to dietary supplementation of *Streptococcus* strain of bacteria (as a source of a - linolenic acid) resulted in reduction of CH:HDL ratio and increase in CH:TG ratio in *Gallus domesticus* (Figure 3).

Daggy *et al.*[26] reported that the dietary PUFA reduce LDL and VLDL cholesterol. It is proposed that n3 PUFA may alter the lipoprotein metabolism. Little increase in liver alkaline phosphatase (data not shown here) and decrease in serum alkaline phosphatase activity (Figure 4) with decrease in liver GOT activity (data not shown here) and insignificant changes in GPT and LDH activity in serum once again confirm the well being state of bird due to dietary supplementation of *Streptococcus* strain of bacteria for 30 days. Little change in alkaline phosphatase activity in liver and serum and GOT activity in liver might be due to shifting of some metabolic pathways (which need to be confirmed in future) in *Gallus domesticus* due to supplementation of *Streptococcus* bacterial strain over a period of 30 days. Olurede and Longe [27] reported the change in the serum GPT activity in chicks due to dietary supplementation of palm oil. It is reported that dietary fatty acids alter the inositol phosphate metabolism and protein Kinase C activity to regulate intracellular signaling system [27] and this might alter the functioning of desaturation system in the endoplasmic reticulum to convert linoleic acid and/or linolenic acid to their respective PUFA.

Summary :

Marine bacteria are known to produce a wide range of compounds, which have potential application as bioactive compounds, probiotics and nutritional supplements. These organisms are now being screened for production of polyunsaturated fatty acids as well as specific fatty acids. In our laboratory we isolated such bacterial isolates (*Streptococcus sp*) from costal sediment, which was having high efficiency (more than 25%) for *de novo* synthesis of alpha – linolenic acid when grown in sodium acetate medium. This bacterial isolate was grown on large scale and was supplemented to the *Gallus domesticus* through commercial feed. The health status of the bird and the protein concentrations of various tissues as a well as their fatty acid profiles and serum lipid profiles were determined. This strain of bacteria acted as growth promoting factor and kept the birds in well being state.

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