Isolation, Screening and Evaluation of Methionine Producing Probiotics on Production Performance of Giriraja Chicks

Nagaraj Naik*, N Jaishankar, Udaykumar Nidoni, Mathad P F, Ambarish G, and S K Nanda

***Abstract*—** Fifty four bacterial isolates were isolated from soil, milk and poultry waste samples. All bacterial isolates were screened for their ability of methionine production. Among them, the most efficient bacterial isolate MM7 was inoculated with maize powder which serves as carrier material in preparation of probiotic poultry feed additive. Further, an experiment was conducted at poultry farm, Bheemarayanagudi using 90 day old Giriraja chicks, The two treatment groups T_1: control with synthetic methionine without Methionine producing probiotics, T_2: with Methionine producing probiotics without synthetic methionine, each treatment contains three replications and each replication contains 15 chicks. The effect of probiotic on chick health. Weekly body weight, Feed consumption, livability were recorded and subjected to statistical analysis. The results indicate that supplementation of methionine producing probiotics improves body weight gains, feed conversion, livability% and better economic returns. Hence, it can be used as supplemental poultry feed for better returns.

***Keywords*—** Giriraja chicks, Isolates, Methionine, Probiotics.

I. Introduction

METHIONINE is the essential sulfur containing amino acid necessary in poultry diets for proper cell growth, egg size and feather development. While methionine is naturally present in organic poultry feed but the amount is not sufficient to maintain the optimal health of the birds. The National Organic Standards Board (NOSB) determined that the loss of the use of synthetic methionine would disrupt the organic poultry market and cause substantial economic hardship to organic poultry operations.

Several reports showed that supplementation of critical animo acids to broiler diets improved the body weight gain and feed efficiency (11, 6). However, chemical production of crystalline methionine is undesirable as it requires hazardous chemicals such as acrolein, methylmercaptan, ammonia, and cyanide. Some of the probiotic microorganisms have the ability to produce methionine *in situ.* Majority of the probiotic products are based mainly on Lactobacillus acidophilus, although other organisms such as Streptococcus faecium, Bacillus subtilis and yeast are also used (2).

Inclusion of probiotics in broiler diets has been proposed to improve growth performance for competitive exclusion of pathogens, to stimulate intestinal immune response and to maintain the balance of micro-organisms in the gastro-intestinal tract of birds (15, 7, 8). It is also reported that the supplemental dietary microbial products function in the digestive system to provide nutrients (10). Certain probiotics have been discovered to facilitate or produce the important nutrient such as lysine (13). However, limited attempts have been made in this direction. In this context, the methionine producing microbes were isolated and the experiment was designed to evaluate the effect of dietary supplementation of a methionine producing probiotics comprising of live microbial cultures over the synthetic methionine on performance of giriraja chicks. The results of the experiment may open up new vistas in utilizing probiotics to cater to the needs of amino acids particularly methionine requirement of the poultry.

II. Materials And Methods

Bacterial isolates were isolated from soil, milk and poultry waste samples by serial dilution and pour plate method. All bacterial isolates were screened for their ability to produce methionine. Chromatography was used for qualitative analysis of methionine whereas acidic ninhydrin method (colorimetric method) was used (3) for quantitative analysis. Among them, one most efficient methionine producing bacterial isolate (MM7) was selected for further study. For the preservation of selected methionine producing bacterial isolate, glycerol stocks were prepared, for which bacterial culture was grown in MRS broth at 30ºC±7 for 16 hours. Then 0.5ml of culture broth was added in vials containing sterilized 500µl glycerol. The glycerol stocks were preserved at -20 ºC. Further bacterial isolate was inoculated with maize powder which serves as carrier material in preparation of probiotic poultry feed.

An experiment was conducted using 90 day old Giriraja

Nagaraj Naik*, Scientist (Microbiology), Department of Processing and Food Engineering, University of Agricultural Sciences, Raichur, 584102, Karnataka, India, (corresponding author's phone : +917795707922, E-mail: naikraj2u2@rediffmail.com)

Jaishankar N, Assistant professor, Department of Animal Science,UAS,Raichur,India.(email:chandu_vet2005@rediffmail.com).

Udaykumar Nidoni Professor, UAS, Raichur, India.

Mathad P F, Ambarish, Assistant Professor, UAS, Raichur. India

S K Nanda, Project Coordinator, AICRP on PHT, PAU, Ludhiana, India

chicks, to evaluate the effect of probiotic poultry feed on its production performance with two groups T_1: control without Methionine producing probiotics, T_2: Methionine producing probiotics (1g/l H_2O). Each treatment contains three replications and each replication contains 15 chicks. The birds were reared on deep little system. The other managemental practices including vaccination programme were accomplished during the 42 day experimental period as per the prevailing commercial practices. The feed was formulated as per NRC 1984 and offered *ad lib*. The body weight of individual chicks and group feed intake of chicks in each replicate were recorded at weekly intervals.

The feed conversion ratio was calculated on the basis of unit feed intake to unit gain in body weight for each replicate, separately. The mortality of birds was recorded as and when occurred. The net return was calculated by taking account of prevailing prices of constituent feed ingredients and additive including methionine producing probiotics on par with synthetic methioine and sale price of live birds at marketing age. The data were subjected to statistical analysis (unpaired two tailed 't' test as per (14). The comparison among means was made by Duncan's multiple range test (4) for significance.

III. Results And Discussion

A. Isolation and screening of methionine producing bacterial isolates

From 54 bacterial strains isolated, 26 were isolated from poultry waste, 20 were isolated from soil and 8 from milk (Table I). Out of fifty four bacterial isolates three were found to be capable of producing methionine (Table II). Among three bacterial isolates, MM7 yielded highest methionine (8.83 mg/ml) and it was inoculated with maize powder to prepare probiotic poultry feed.

B. Effect of methionine producing probiotic on production performance of chicks

The weekly body weight gains, Feed consumption, Feed conversion ratio, livability percent and the economic returns were present in Table III to Table VI respectively as follows. The weekly body weight gains (g) varies non significantly between the treatments whereas the cumulative body weight gains varies significantly ($P < 0.05$) from 883 g (without methionine producing probiotics) to 955.7 g (with methionine producing probiotics). Same results were observed by Rezaei (11) when fed probiotics in case of broilers. The increase in body weight gains may be due to the better health due to beneficial microbes.

The weekly and cummulative feed intake presented in table IV. The cumulative feed consumption (g) varies non significantly ($P>0.05$) from 1747.7g (T2) to 1789.3g (T1). The feed intake increase in methionine producing probiotics, this might be due to increase in digestibility of nutrients contributed by harmless microbes. The cummulative feed conversion ratio varied non significantly ($P>0.05$) from 1.86 (T2) to 2.06 (T1). Probiotics supplemented diets showed increased body weight gains and decreased feed consumption, therefore the feed conversion ratio was better in probiotics supplemented diets. The weekly feed conversion ratio presented in table V.

The cumulative livability percent varies non significantly ($P>0.05$) from 97 (T1) to 100 percent (T2). The major function of probiotics is competitive for adhesion in gastro intestinal track with harmful microbes, hence has benefits to prevent occurrence of disease and improves immunity. Therefore the livability percent was achieved to 100 percent in diet supplemented with methionine producing probiotics.

The net returns was calculated and subjected to statistical analysis, there was statistically significant ($P<0.05$) and net returns varies from Rupees 36.16 (T1) to Rupees 45.80 (T2) per chick. Feeding of methionine producing probiotics has improved body weight gains, reduced feed consumption and improved livability, which has contributed for the better net returns. Similar results were observed by (1, 12, 5) upon feeding of probiotics in broilers.

TABLE I
SCREENING OF BACTERIAL ISOLATES FOR THE PRODUCTION OF METHIONINE

Sl.No	Source of isolation	Code No of Isolate	Methionine Production
1	Poultry Waste	PWM1	-ve
2	Poultry Waste	PWM2	-ve
3	Poultry Waste	PWM3	-ve
4	Poultry Waste	PWM4	-ve
5	Poultry Waste	PWM5	**+ve**
6	Poultry Waste	PWM6	-ve
7	Poultry Waste	PWM7	-ve
8	Poultry Waste	PWM8	-ve
9	Poultry Waste	PWM9	-ve
10	Poultry Waste	PWM10	-ve
11	Poultry Waste	PWM11	-ve
12	Poultry Waste	PWM12	-ve
13	Poultry Waste	PWM13	-ve
14	Poultry Waste	PWM14	-ve
15	Poultry Waste	PWM15	-ve
16	Soil	SM1	-ve
17	Soil	SM2	-ve
18	Soil	SM3	-ve
19	Soil	SM4	-ve
20	Soil	SM5	-ve
21	Soil	SM6	-ve
22	Soil	SM7	-ve
23	Soil	SM8	-ve
24	Soil	SM9	-ve
25	Soil	SM10	-ve
26	Soil	SM11	-ve
27	Soil	SM12	-ve
28	Milk	MM1	-ve
29	Milk	MM2	-ve
30	Milk	MM3	-ve
31	Milk	MM4	-ve
32	Milk	MM5	-ve
33	Milk	MM7	-ve
34	Milk	MM8	**+ve**
35	Milk	MM9	-ve
36	Soil	SM13	-ve
37	Soil	SM14	-ve

38	Soil	SM15	-ve
39	Soil	SM16	-ve
40	Soil	SM17	-ve
41	Soil	SM18	-ve
42	Soil	SM29	-ve
43	Soil	SM20	-ve
44	Poultry Waste	PWM16	-ve
45	Poultry Waste	PWM17	**+ve**
46	Poultry Waste	PWM18	-ve
47	Poultry Waste	PWM19	-ve
48	Poultry Waste	PWM20	-ve
49	Poultry Waste	PWM21	-ve
50	Poultry Waste	PWM22	-ve
51	Poultry Waste	PWM23	-ve
52	Poultry Waste	PWM24	-ve
53	Poultry Waste	PWM25	-ve
54	Poultry Waste	PWM26	-ve

TABLE II
QUANTITATIVE ESTIMATION OF METHIONINE PRODUCTION BY BACTERIAL ISOLATES

Sl.No.	Isolate	Methionine (mg/ml)
1	PWM 5	3.27
2	PWM 17	5.42
3	MM 7	8.83

TABLE III
WEEKLY AND CUMULATIVE BODY WEIGHT GAINS (G) OF GIRIRAJA CHICKS 0-42 DAYS

Treatment	I week	II week	III week	IV week	V week	VI week	Cumulative
T1	40.0±2.7	68.3±5.2	124.0±3.6	127.7±5.8	236.7±18.5	286.0±17.8	883.0±4.7[a]
T2	40.3±1.5	77.0±0.6	124.3±0.9	153.0±10.7	268.0±9.5	293.7±11.4	955.7±9.7[b]
p value	0.92	0.17	0.933	0.106	0.207	0.735	0.003
t value	0.11	1.64	0.090	2.082	1.504	0.363	6.744

TABLE IV
WEEKLY AND CUMULATIVE FEED CONSUMPTION (G) OF GIRIRAJA CHICKS 0-42 DAYS

	I week	II week	III week	IV week	V week	VI week	Cumulative
T1	81.3±4.1	132.3±15	257.3±11.7	299.7±2.4	426.3±9.8	592.7±13.3	1789±30.8
T2	77.3±5.8	140.0±10	244.7±16.9	292.7±2.6	415.3±2.7	578.0±1.5	1747±15.0
p value	0.60	0.69	0.571	0.119	0.342	0.336	0.291
t value	0.56	0.41	0.617	1.976	1.078	1.094	1.217

TABLE V
WEEKLY AND CUMMULATIVE FEED CONVERSION RATIO OF GIRIRAJA CHICKS 0-42 DAYS

	I week	II week	III week	IV week	V week	VI week	Cumulative
T1	2.04±0.0	1.97±0.3	2.07±0.07	2.35±0.10	1.83±0.18	2.08±0.08	2.06±0.05
T2	1.94±0.1	1.81±0.1	1.97±0.13	1.93±0.14	1.55±0.07	1.97±0.08	1.86±0.05
p value	0.62	0.68	0.525	0.073	0.229	0.406	0.051
t value	0.52	0.43	0.696	2.419	1.417	0.928	2.765

TABLE VI
CUMMULATIVE LIVABILITY % AND ECONOMICS OF GIRIRAJA CHICKS FOR 0-42 DAYS

Treatment	LIVABILITY %	ECONOMICS (Rupees)
T1	97.7±2.3	36.16±0.92[a]
T2	100.0±0.0	45.80±1.21[b]
p value	0.374	0.003
t value	1.000	6.345

IV. SUMMARY AND CONCLUSION

Microbes were isolated from poultry waste, soil and milk. Among these microbes isolated from the milk MM7 is capable of producing highest methionine invivo (8.83mg/ml). MM7 was inoculated with maize power to prepare probiotic poultry feed additive. Further feeding such Probiotics as feed additive to replace synthetic methionine reduced feed consumption T2 (1747.7±15.0) from T1 (1789.3±30.8) and the body weight gain increased significantly from T2 (955.7±9.7[b]) than control group T1 (883.0±4.7[a]). Since the feed consumption is less and body weight gain was increased in methionine producing probiotics, the feed conversion ratio was better and non significant in T2 (1.86±0.05) when compared to T1 (2.06±0.05). the livability percent was 100% in probiotics supplemented groups and 97.7% in control group. Hence probiotics improves health and livability. The economic returns were statistically significant in methionine probiotics supplemented groups than control group. The results indicate that supplementation of methionine producing probiotics improves body weight gains, Feed conversion, livability% and better economic returns. Hence, addition of methionine producing probiotics as feed supplement didn't show any adverse effect, therefore can be used as supplemental poultry feed to replace synthetic methionine.

REFERENCE

[1] Chiang, S. H. and W. M. Hsieh, 1995. Effect of direct fed microorganism on broiler growth performance and litter ammonia level. Asian-Aust. J. Anim. Sci., 8:159-162.

[2] Cheeke, P. R., 1991. Applied Animal Nutrition: Feeds and Feeding. MacMillan Publishing Company, New York, USA.

[3] Chinard, F. P.,1952. Paper Chromatography *J. biol. Chem.* 199: 91.

[4] Duncan D B. 1955. Multiple range and F-tests. *Biometrics* **11**: 1-42.

[5] Fuller, R., 2001. The chicken gut microflora and probiotic supplements (Review). J. Poult. Sci., 38: 189-196.

[6] Garcia A and Batal A B. 2005. Changes in the digestible lysine and sulfur amino acid needs of broiler chicks during the first three weeks post-hatching. *Poultry Science* **84**: 1350-1355.

[7] Kobayashi Y, Koike S, Taguchi H, Itabashi H, Kam D K and Ha J K. 2004: Recent advances in gut microbiology and their possible contribution

to animal health and production- A review. *Asian-Australian Journal of Animal Science* **17**(6):877-884.

[8] Koraoglu M and Durdag H. 2005. The influence of dietary probiotics (*Saccharomyces cerevisiae*) supplementation and different slaughter age on the performance, slaughter and carcass properties of broilers. *International Journal of Poultry Science* **4**(5): 309-316.

[9] NRC. 1984. Nutrient requirements of poultry. 8th edn. National Academy Press. Washington D.C., USA.

[10] Owings W J, Reynoldas D L, Hasiak R J and Ferket P R. 1990. Influence of dietary supplementation with Streptococcus faecium M - 74 on broiler body weight, feed conversion, carcass characteristics, and intestinal microbial colonization. Poultry Science 69: 1257-1264.

[11] Rezaei M, Nassiri Moghaddam H, Reza J P and Kermanshahi H. 2004. The effect of dietary protein and lysine levels on broiler performance, carcass characteristics and N excretion. *International Journal of Poultry Science* **3**(2): 148-152.

[12] Santoso, O. U., K. Tanake., S. Ohtani and M. Sakaida, 2001. Effect of fermented product from Bacillus subtilis on feed conversion efficiency, lipid accumulation and ammonia production in broiler chicks. Asian-Aust. J. Anim. Sci., 14: 333-337.

[13] Shaw-Reid C A, McCormick M M, Sinskey A J and Stephanopoulos G. 1999. Flux through the tetrahydrodipicolinate succinylase pathway is dispensible for L-lysine prouduction in *Corynebacterium glutamicum*. *Journal of Applied Microbiology and Biotechnology* **51**: 235-333.

[14] Snedecor G W and Cochran W G. 1989. Statistical Methods. 8th Edn., Iowa State University Press, Ames, Iowa.

[15] Wenk C. 2000. Recent advances in animal feed additives such as metabolic modifiers, antimmicrobial agents, probiotics, enzymes and highly available minerals. *Asian-Australian Journal of Animal Science* **13**: 86-95.