Isolation of Methionine Producing Probiotics and Evaluation on Egg Production Parameters in Commercial Layers

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

***Abstract*—** The microbes were isolated from poultry waste, soil and milk. Among these microbe isolated from milk MM7 capable of producing highest methionine invivo (8.83mg/ml). MM7 strain was inoculated with maize powder to prepare probiotic poultry feed additive. Further, An experiment was conducted with 23 weeks old commercial layers to assess the effect of the methionine producing probiotics on egg production parameters in white leghorn layers, The two treatment groups T_1: control with synthetic methionine without Methionine producing probiotics and T_2: with Methionine producing probiotics replacing synthetic methionine, each treatment contains three replications and each replication contains four layers. An attempt was made to use probiotics culture MM7 having $88X10^4$cfu/g producing 1.12g of synthetic methionine invivo to replace synthetic methionine. The results shows that the feed consumption, egg weight, egg shape index, albumen index, haugh unit score, egg yolk color, yolk index, shell thickness, feed conversion ratio per kg egg mass, feed efficiency per dozen egg and egg: feed price ratio were statistically non significant between the treatments. Hence, methionine producing probiotics can be used to replace synthetic methionine as supplemental poultry feed for better returns.

***Keywords*—** Methionine, Probiotics, Egg, White leghorn, layers.

I. Introduction

PROBIOTICS include viable microbial and microbial fermentation products which are beneficial to decrease the undesirable microflora population in the gastro- intestinal tract of chicks [3] and build-up resistance against diseases by stimulating the immune system [4], [12]. 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 [4].

The protein quality and quantity in poultry diets assumes great significance. The efficiency of protein utilization in layers depends to a large extent on the amino acid composition of diet, the egg production parameters improved by feeding critical amino acids [6], [2]. Methionine and lysine are the first and second limiting amino acids in layer diets [9] respectively leading to their adjudication either from synthetic source or feed stuffs *perse*. However, normally a practical layer diet is usually deficit in both methionine and lysine (critical amino acids). The supplementation of amino acids in a low protein diet has been found to increase egg mass, egg weight and feed conversion in a sunflower seed cake based diet [8].

The deficient amount of amino acid methionine must be supplemented in the form of synthetic methionine or any probiotic preparation, which can specifically synthesise adequate amount of methionine in the gut of the bird by utilizing the prevalent nitrogen. In addition to this, probiotics such as Lactobacillus species [1] in layer diets did positively influence hen day egg production, FCR, egg weight and albumen. Diet formulated with sub optimal levels of sulphur amino acids and lysine along with direct fed microbials performed equal to those fed with adequate amino acids. Keeping in view the afore said facts, a comparative study was designed to put on record the effect of methionine producing microbes, which can produce methionine in the gut of the birds sufficient enough to meet the requirement. And this was compared with that of synthetic methionine in 23 weeks old commercial layers.

II. Materials And Methods

Microbes will be isolated from poultry waste, soil and milk, then screened for methionine production in vitro, the highest methionine producing microbes were used for preparation of probiotic poultry feed additive. Further, the experiment conducted with twenty four commercial layers of 23 weeks old to compare between two treatments. T1- control diet meeting methionine using synthetic methionine, T2- diet with methionine producing probiotics (synthetic methionine was replaced by methionine producing probiotics), and each treatment contains three replications and each replication contains four layer birds. The birds were reared on cage system. The isolated microbial culture having $88X10^4$cfu/g of feed producing 1.12g of synthetic methionine invivo, therefore methionine producing probiotics was used to replace synthetic methionine. The other managemental practices including vaccination Programme were accomplished during

Jaishankar, N., Assistant professor, Department of Animal Science, UAS, Raichur, India. (corresponding author's phone:9620025960; e-mail:chandu_vet2005@rediffmail.com).

Nagaraj Naik, Scientist, UAS, Raichur, India (e-mail: naikraj2u2@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

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the experimental period as per the commercial practices. The feed was formulated as per NRC 1984. 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 egg mass and also feed efficiency per dozen eggs for each replicate calculated separately. The haught unit score, albumen index, yolk index were calculated. The mortality of birds was recorded as and when occurred. The economics was calculated in terms of egg: feed price ratio (EFPR). The data were subjected to statistical analysis (unpaired two tailed ‘t’ test) as per Snedecor and Cochran [14]. The comparison among means was made by Duncan’s multiple range test [5] for significance.

III. Results And Discussion

Fifty four bacterial strains isolated; 26 were isolated from poultry waste, 20 were isolated from soil and 8 from milk. Out of fifty four bacterial isolates three were found to be capable of producing methionine PWM 5, PWM 17 & MM7. Among three bacterial isolates, MM7 yielded highest methionine (8.83 mg/ml) and it was inoculated with maize powder to prepare probiotic poultry feed.

The layer birds with uniform body weight was selected for the experiment and the body weight of layer chicks ranges from 1375 g to 1450 g. the Average feed consumption was statistically non significant and the data from 23rd week to 26th week is presented in Table I. The feed consumption was gradually increased every week.

TABLE I
Average Feed Consumption, Egg Production% And Egg Weight (G)

	23rd week	24th week	25th week	26th week
Average Feed Consumption (g)				
T1	82.5±1.4	97.5±1.4	103.3±1.7	104.7±1.7
T2	82.8±1.2	95.8±0.8	102.5±3.8	104.3±2.3
p value	0.866	0.374	0.851	0.912
t value	0.180	1.000	0.200	0.118
Egg Production %				
T1	75.5±0.6	86.7±0.7	91.2±0.9	95.8±0.7
T2	75.8±0.3	86.8±0.9	91.8±0.7	95.8±0.3
p value	0.643	0.890	0.579	1.000
t value	0.500	0.146	0.603	0.000
Egg Weight (G)				
T1	45.5±1.2	46.5±0.8	48.4±1.8	47.7±1.1
T2	45.9±0.3	48.0±0.4	50.2±2.5	50.4±1.2
p value	0.790	0.201	0.599	0.185
t value	0.285	1.528	0.570	1.602

The egg production was increased gradually every week from 75.5 percent to 95.8 percent, the production performance was on par with the synthetic methionine. But the weekly egg production was statistically non significant and presented in table I. The egg weight (g) varied non significantly between the treatments, but numerically the diet with methionine producing probiotics have shown increased egg weight. The weekly egg weights were presented in table I.

The egg shape index calculated by considering length and width of an each egg using vernier calipers [13] and expressed in percent. The egg shape index varied non significantly between the treatments, which indicates that the methionine producing probiotics is performing on par with synthetic methionine. The results of egg shape index presented in table II.

TABLE II
Egg Shape Index %, Albumen Index And Yolk Index

	23rd week	24th week	25th week	26th week
Egg Shape Index %				
T1	134.4±1.4	131.5±1.5	126.5±6.2	130.7±1.3
T2	135.1±0.9	132.1±1.9	127.9±1.3	128.0±1.7
p value	0.709	0.808	0.838	0.290
t value	0.400	0.259	0.218	1.220
Albumen Index				
T1	10.4±0.7	10.2±0.6	12.0±0.4	13.1±0.4
T2	11.9±0.8	11.5±1.0	12.7±0.4	13.3±0.3
p value	0.230	0.313	0.259	0.664
t value	1.417	1.155	1.316	0.468
Yolk Index				
T1	42.6±0.5	40.6±0.2	40.1±1.0	39.7±0.7
T2	42.7±0.6	40.8±1.0	40.8±0.1	40.0±0.9
p value	0.903	0.838	0.515	0.855
t value	0.130	0.218	0.714	0.195

The albumen index calculated by considering the height and diameter of egg albumen measured using ames haugh unit spherometer and vernier caliper. The albumen index varied non significantly between the treatments and the weekly albumen index results presented in table II. The results were on par with the synthetic methionine.

The yolk index calculated by taking the height and diameter of the yolk measured using ames haugh unit spherometer and vernier calipers. The yolk index varied non significantly between the treatments and the results were presented in table II.

The yolk color of every egg was scored by matching technique using roche yolk colour fan [15], its color value denotes the color intensity from 1 to 14 scale according to the degree of color. In the layer diet maize was replaced by broken rice therefore the yolk color was less than scale 2 and the results were non significant between the treatments (table III).

TABLE III
YOLK COLOR, SHELL THICKNESS (MM) AND FEED CONVERSION RATIO PER KG EGG MASS

	23rd week	24th week	25th week	26th week
Yolk Color				
T1	1.67±0.17	1.83±0.17	1.83±0.17	1.83±0.17
T2	1.67±0.17	1.83±0.17	1.83±0.17	1.83±0.17
p value	1.000	1.000	1.000	1.000
t value	0.000	0.000	0.000	0.000
Shell Thickness (mm)				
T1	0.29±0.0	0.29±0.0	0.32±0.0	0.31±0.0
T2	0.29±0.0	0.30±0.0	0.32±0.0	0.31±0.0
p value	0.519	0.561	0.492	1.000
t value	0.707	0.633	0.756	0.000
Feed Conversion Ratio Per Kg Egg Mass				
T1	2.37±0.05	2.26±0.17	2.14±0.08	2.20±0.08
T2	2.39±0.05	2.17±0.21	2.04±0.03	2.07±0.09
p value	0.816	0.746	0.333	0.383
t value	0.248	0.348	1.101	0.978

The shell thickness measured using digital [11]. The shell pieces devoid of shell membranes at broad end, narrow end and middle band were carefully selected and measured the shell thickness. The results were statistically non significant and presented in table III.

The feed conversion ration per kg egg mass was calculated by considering the feed intake, egg weight and egg production. The results varied non significantly between the treatments. The data presented in table III. Methionine producing probiotics performed on par with the synthetic methionine. The feed efficiency per dozen eggs between treatment varied non significantly and the data presented in table IV.

TABLE IV
FEED EFFICIENCY PER DOZEN EGGS, HAUGH UNIT SCORE AND EGG FEED PRICE RATIO (EFPR)

	23rd week	24th week	25th week	26th week
Feed Efficiency Per Dozen Eggs				
T1	1.32±0.02	1.30±0.11	1.24±0.02	1.26±0.02
T2	1.32±0.02	1.28±0.12	1.23±0.05	1.25±0.03
p value	1.000	0.910	0.851	0.912
t value	0.000	0.121	0.200	0.118
Haugh Unit Score				
T1	89.0±2.4	88.9±1.3	92.1±0.7	94.4±1.7
T2	92.6±2.0	91.1±2.3	92.1±1.0	95.9±1.0
p value	0.319	0.464	0.961	0.481
t value	1.137	0.810	0.052	0.777
Egg Feed Price Ratio (EFPR)				
T1	1.48±0.03	1.53±0.12	1.57±0.03	1.55±0.02
T2	1.56±0.03	1.64±0.14	1.67±0.06	1.64±0.04
p value	0.107	0.590	0.204	0.119
t value	2.08	0.586	1.518	1.981

The relationship between egg weight and albumen height for each egg was calculated as haugh unit score as per [7], the haugh unit score was statistically non significant and the results were presented in table IV.

The egg feed price ratio is used to find out the ratio between the receipt from egg and expenditure on feed. The EFPR varies non significantly from 1.47 to 1.59. The result in diet with methionine producing probiotics was better than diet with synthetic methionine. The results were presented in table IV. The livability of the birds was 100 percent in both the treatments.

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 of Methionine producing Probiotics as feed additive to replace synthetic methionine has performed better than synthetic methionine. The weekly feed consumption, egg weight, egg shape index, albumen index, haugh unit score, egg yolk color, yolk index, shell thickness, feed conversion ratio, egg: feed price ratio, livability percent were non significantly varied between the treatments. The results indicate that supplementation of methionine producing probiotics improves 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.

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