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Research Article

Application Trial of L-Lysine on Chicks by Microbial Fermentation Using Different Bacterial Strains

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ABSTRACT

L-lysine, one of the well-known essential amino acid is in great demand as medicament and as an additive to animal feed or human food stuff. The importance of L-lysine as an essential amino acid in the nutrition of human beings has made it desirable supplement of the diet in recent years. For these reasons, efforts are now being focused on the potential of L-lysine derived bacteria and its application. In the present study, the production of L-lysine was achieved through fermentation developed from locally isolated bacterial strains. For *Bacillus subtilus* 23.3 g/L, for *Streptococcus sp.* 23.4 g/L and for *Bacillus sp.* 21.7 g/L of lysine was produced correspondingly. The application of L-lysine on chicks indicated that the L-lysine produced in a crude form through fermentation using bacterial strain has sufficient enough to increase the biomass of chicks *i.e.,* for *Bacillus subtilus* weight gain was 44 g for *Streptococcus sp.* and *Bacillus sp.* it was 48 g. The cost estimation of the product formed in the present study is Rs.43.2 per kg while the price of available commercial feed is Rs.50.0 per kg. The tremendous results indicating that the product efficiency is much higher than the results of commercial feed.

Keywords: Chick feed; Lysine; Bacterial strain; Fermentation product; Application

INTRODUCTION

Globally, the use of L-lysine as an essential amino acid is exercised extensively in articulating diets for poultry. For this, application of lysine in chick feed 'or as the limiting amino acid' is plaid by the addendum of L-lysine into the pragmatic poultry diets, that eventually stipulates the ways for escalating the efficacy of protein deployment plus environmental nitrogen secretion will be abridged. Freshly, fodder makers stay confronted by means of a cumulative array of liquid artifacts comprising methionine as well as choline and consequently may also integrate free fluctuating liquid lysine artifact into their fodder mingling stratagems. Several analytical approaches like weight gains of fledgling chicks are wrought to appraise the dietary response of lysine in feed [1-5].

In developing countries people are consuming cereal based foodstuffs that are deficient in lysine therefore, protein intake needs to be enhanced (Figure 1). Also, the recent status needs to augment the current diet with lysine so as to execute the dietary necessities for the furtherance of human health as well as the domestic meat giving animal included fowl and fisheries. Specifically, inside the fowl trade application of vegetables as well as plant centered constituents for devising of fowl provender lacks amino acids as, plant proteins remain poor in vital amino acids mainly lysine.

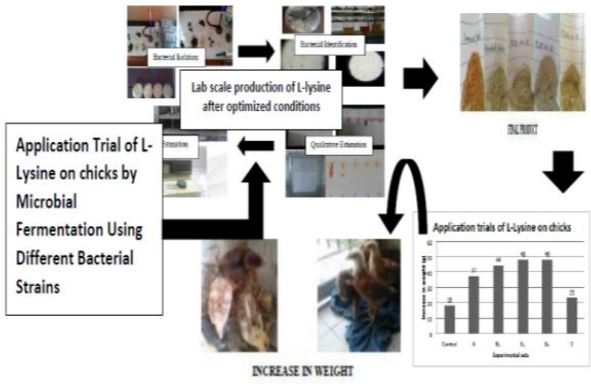

Figure 1: Graphical abstract.

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Seeing that Pakistan is an emerging land, massive quantity of imported trade has been consuming in the importation of such an indispensable amino acid needed for forage trade. During 2015, the global market demand for L-lysine was about 2.2 million tons per year. Therefore, the present work is planned to maximize yields of free lysine obtained in a culture broth by using different bacterial strains recover from various soil and water samples. Experiment design also focuses on the characterization and optimum growth parameters along with its applications in chick feed [6-10].

MATERIALS AND METHODS

Plant material: The plant utilized for the study of L-lysine application is *Pennisetum glaucum* (L.)R. Br. commonly known as pearl millet. The pearl millet is widely grown millet. Millets are an assembly of highly erratic small-seeded grasses, extensively grown around the sphere as cereal crops and grains for fodder as well as human food. Pearl millet is easily available and cheap crop as well as very important for the growing economy of Pakistan. For the experimental set, it is purchased from the readily available local market and then used for application trials.

Commercial feed: Commercial feed contains different form of artificial fodder easily available in local markets. For L-lysine application trials, poultry feed purchased from the local Tollinton Market, Lahore, was used. The composition of such feed was unknown and the suppliers of the feed retained the constituents confidential [11-16].

Lab scale production of L-lysine after optimized conditions: Mass production of L-lysine was accomplished at lab scale under optimized conditions. The parameters considered were as follows:

Organism: Three finest performed bacteria were exhausted for further study. These were identified as:

- PCSIR-NL-37 as *Bacillus subtilus.*
- PCSIR-NL-42 as *Streptococcus sp.*
- PCSIR-NL-45 as *Bacillus sp.*

Cultivation: Each flask containing assay medium was inoculated with optimized inoculum size and incubated in an incubator at their optimized temperature for optimized incubation hours correspondingly.

Qualitative estimation: Qualitative estimation of the product was accomplished using chromatographic techniques. Paper chromatography explained in the detection of lysine during preliminary screening test, followed by thin layer chromatography was depicted for this approximation.

Quantitative estimation: Quantitative estimation of the product was done by following the assay procedure. Optical density was logged and the extent of lysine was calculated.

Production of feed for application trials: The L-lysine obtained from the supernatant was autoclaved at 121°C and 15 lb. pressure for 5 minutes to dispatch the bacterial cells. Then the product was filtered and calculated the respective amount of lysine benevolence. For the production of feed, vigorous expanse of L-lysine produced in a culture broth was purloined and then jumbled with the plant material i.e. *Pennisetum glaucum* (L.)R. Br [17-22]. This intermingled material was daubed together for 2 days till the seeds of the plant utterly engross the product. When the seeds are absolutely soaked in the product, it was spread on a piece of newspaper and consigned in open air for drying. After 2 days the experimental feed was ready for trial usage (Figure 2).

Figure 2: Application of l-Lysine.

Application trial of L-lysine: Application of L-lysine formed above can be governed by designing a modest and effectual experiment. The experiment fabricated in such a way as to attain maximum positive outcome and minimizing the gambles. The experiment was conceded in the lab using Hybrid chicks of almost same age and gender (male, approx. 2 months older). The efficacious maneuvering of the experiment may un-cluttered the new doors in the betterment of poultry industry in Pakistan. The fruitful results can apprehend the usage of commercial feed that is most of the time crammed with harmful hormones which will ultimately menacing for chicks as well as human health [23-30].

The formed product is devoid of any toxic pathogen as well as detrimental chemical. The final product employed for the experiment was upheld and checked regularly for any sort of contamination. Following were the parameters taken into consideration for the experiment design:

- Three trials of experiment were executed in which each trial encompasses 15 days surveillance.
- Maximum amount of lysine was curbed in available literature (3 g/kg of feed) and safe levels of lysine in sustenance of chicks were conserved.
- The chicks were fed with their respective feed and care was executed for the incorporation of any other edible stuff.

- Experiment consists of 18 chicks in 6 different sets appropriately labeled (Table 1).
- Each set comprised of 3 chicks fed on their respective dosage source.

Table 1: Experimental design for the application trial of l-lysine produce.

Serial No.	Experimental sets	Dosage source	No. of chicks	Labeling color
1	Control	*Pennisetum glaucum* (L.) R. Br.	3	Orange
2	A	Standard L-lysine solution (500 mg/mL)	3	White
3	B_1	PCSIR-NL-37 as *Bacillus subtilus*	3	Green
4	B_2	PCSIR-NL-42 as *Streptococcus sp.*	3	Red
5	B_3	PCSIR-NL-45 as *Bacillus sp.*	3	Purple
6	C	Commercial feed	3	Black
Total			18	

Weights of chicks were contemplated before and after the completion of each trial respectively. Growth parameters were perceived and deliberated the weight gain as well. Weight gain was basically calculated by subtracting the initial weights from the final weights of the chicks.

Difference in the body weights of experimental sets were calculated and inscribed in the form of a table. Also, any sort of behavioral change during study was logged and observed thoroughly.

RESULTS

The application trials of L-lysine on chicks by microbial fermentation using different bacterial strains were conceded in Food and Biotechnology Research Centre (FBRC), Lahore. The rampant investigation was consummate and deliberated scrupulously.

Laboratory scale production of L-lysine after optimized conditions: Laboratory scale production of L-lysine was carried out after optimizing all growth requiring conditions. The quantitative estimation is given in Table 2. For *Bacillus subtilus* the optimized parameters result in 23.3 g/L yield of lysine. For Streptococcus sp. the parameters for optimization give rise to 23.4 g/L of lysine. For *Bacillus sp.* the parameters for optimization bring about 21.7 g/L yield of lysine [32].

Table 2: laboratory scale production of l-lysine.

Strain (PCSIR-NL-NO.)	Opt. Subs.	Opt. Temp.	Opt. Met.	Opt. pH	Opt. I.P.	Opt. I.S.	Bacterium	l-lysine produced (g/l).	Standard deviation
37	GMV	45°C	Fe	6	96 h	0.3 ml/L	*Bacillus subtilus*	23.3 ± 1.09[a]	1.89
42	MMV	40°C	Mg	6.5	96 h	0.3 ml/L	*Streptococcus sp.*	23.4 ± 0.93[a]	1.616
45	GMV	30°C	Mg	5.5	216 h	0.2 ml/L	*Bacillus sp.*	21.7 ± 1.33[a]	2.309

Significance level=0.05

Error mean square=3.84

Degree of freedom=6

LSD 0.05=3.91

MMV: Molasses Media with Vitamins, GMV: Glucose Media with Vitamins

OPT.=Optimum, SUBS.=Substrates, TEMP.=temperature, MET.=Metal Ion,

I.P.=Incubation Period, I.S.=Inoculum Size.

Application of L-lysine: L-lysine produced during investigation was applied on chicks to check its effects on weights. (Table 3) shows the weights (mean) of chicks before and after the completion of each trial. Wight gain of control set is 18 g while weight gain of experimental sets B_1, B_2 and B_3 are 44 g, 48 g and 48 g respectively. On the other hand, the weight gain of set A and set C is 37 g and 23 g respectively. Difference in the body weights of experimental sets is calculated and inscribed. Also, a behavioral change of experimental set B_1 during study is logged and observed thoroughly. Difference in the weight gains of experimental sets shows the capability of the product formed as the crude form of lysine.

Table 3: Application trials of l-lysine on chicks.

Serial no.	Experimental Sets	Initial Weight (g_1)	Final Weight (g_2)	Weight Gain (g_2- g_1)	Behavioural Change
1	Control	90 ± 10.39^{c}	108 ± 10.39^{c}	18	normal
2	A	115 ± 21.3^{ab}	152 ± 21.3^{ab}	37	normal
3	B_1	132 ± 25.4^{ab}	176 ± 25.4^{ab}	44	Recovered quickly after injury
4	B_2	140 ± 27.7^{ab}	188 ± 27.7^{a}	48	normal
5	B_3	142 ± 27.7^{ab}	190 ± 27.7^{a}	48	normal
6	C	95 ± 13.2^{b}	118 ± 13.2^{ab}	23	normal

Significance level=0.05

Error mean square=1799.11

Degree of freedom=24

LSD 0.05=71.477

The experimental results shown in (Figure 3) indicated that the L-lysine produced in a crude form through fermentation using bacterial strain has sufficient enough to increase the biomass of chicks. Results also indicating that the product (experimental sets B_1, B_2, B_3) has greater efficiency than the available commercial feed (experimental set C) in increasing the weights of chicks.

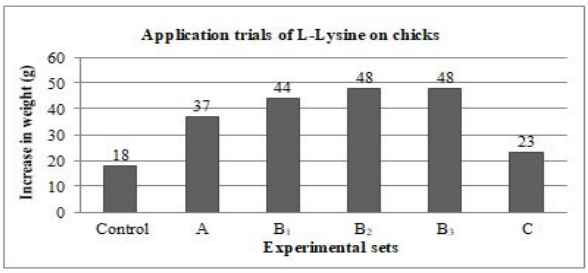

Figure 3: Graphical representation of weight gain of chicks.

Cost analysis for raw material: The cost of production for the experimental feed was much less than the cost of available commercial feed (Table 4). Based on the average amount of L-lysine produced by glucose media (average of all three is 22.8 g/L) the cost is Rs.26.28. The results obtained by experimental feed are tremendous, opening a new era in the production of feed for poultry industry.

Table 4: Analysis of the cost of raw materials used for the production of l-lysine.

Raw material	Price (rs. /kg)	Amount used (in g)	Cost in (rs.
Glucose	105	60	6.3
Yeast extract	500	5	2.5

Peptone	210	5	1.05
Potassium di-hydrogen Phosphate	95	0.75	0.07
di-potassium hydrogen phosphate	103	0.75	0.07
Ammonium sulfate	8	23	0.18
Calcium carbonate	10	10	0.1
Magnesium sulfate	5	1.545	0.007
Manganese sulfate	5	0.058	0.0002
Sodium chloride	25	1.25	0.03
Urea	16	2.5	0.04
Vitamin source	**Price and No. oftablets**	**Usage (g)**	**Cost (Rs.)**
Becefol Tablet	Rs.70 with 25 tablets	2.1	2.8

Average amount of L-lysine produced of medium (g/l)=22.8

Cost of raw materials for the production of 0.5 kg of L-lysine=Rs.13.14

For 1 kg of lysine=13.14 x 2=26.28

- The price for each of the materials was based on value obtained from local market.
- The amount used in the calculation was based on the laboratory scale experiment described in materials and methods of the current report.

DISCUSSION

In the present study Laboratory scale production of lysine is carried out. For *Bacillus subtilus* the optimized parameters result in 23.3 g/L yield of lysine. For *Streptococcus sp*. the parameters for optimization results in 23.4 g/L of lysine. And for *Bacillus sp*. the parameters for optimization results in 21.7 g/L yield of lysine respectively. The most interesting feature of the present study was the yield of L-lysine. Some of the previous reports on lysine production, using different bacterial strains, different media composition and different growth parameters reflect highly variable results. From the results of the laboratory scale study, conclusion can be drawn that locally available cane molasses is a useful carbon source along with commercially available glucose.

For industrial production of L-lysine the choice of raw materials depends largely on economic considerations. This does not mean simply the cost of raw materials, but also the cost for isolation and purification of fermentation broth as well as various considerations concerning fermentation yield, fermentation hours, and treatment of waste materials produced by fermentation and so on. Similarly, the optimization of cultural conditions in relation to oxygen supply and carbon dioxide removal has played a key role in the scale up and commercial production of L-lysine. These factors determine both the rate of cells growth and product formation. In the present study, a simple analysis of the cost of the raw materials used for the production of L-lysine is presented. Based on the average amount of L-lysine produced by glucose media (average of all three is 22.8 g/L) the cost is Rs.26.28. The quantity of L-lysine produced by such bacteria as well as the end products is quite comparable. Thus, keeping in view, the high cost of other carbon sources, commercially available glucose justifies its use as a carbon source for L-lysine fermentation. Similarly, the use of becefol tablet and ammonium sulfate are also justified because of the high cost of biotin, L-leucine and malt extract respectively. Hence, a considerably less expensive L-lysine fermentation is carried out using these raw materials. Keeping in view the high cost of organic nutrients, such as yeast extract, peptone, and meat extract, they were replaced by low cost and locally available raw materials such as commercial grade glucose, corn steep liquor, soy bean protein acid hydrolysate and fish meal respectively. Concentrations of inorganic salts were kept similar in all media to those used in shake flask experiments. Cane molasses are generally used as carbon source in the industrial production of L-lysine. However, cane molasses contains many constituents which are toxic to bacterial growth. Therefore, superphosphate treatment was done to remove the toxic substances in the form of heavy precipitate.

Mostly the final product is usually presented as a salt, Lysine-HCl (Lysine mono-chloridrate). However; it can also be presented as L-Lysine liquid formulations or in granulated form. Consequently, it would be imperative to increase lysine production by utilizing all the available cheap and non-conventional lysine-yielding resources (agriculture and industrial waste). In the present study L-lysine is produced in a liquid form as a final product by utilizing the available and cheap resources.

The application of L-lysine produced is checked by performing a simple experiment on chicks. As governed the digestible lysine necessity of chicks with 1-15 days old under 2 trials. In his Investigation of d-lysine necessity with 1-14 days old during trial

one showed a quadratic wrecked-mark cutoff point as 1.27 meant for physique weightiness increase; however, during trial two, it's displayed as 1.18 meant for physique weightiness increase as well as 1.261 for food alteration.

The final product of the present study employed for the experiment is upheld and checked regularly for any sort of contamination. Three trials of experiment are executed in the present study from which each trial encompasses 15 days surveillance. Also, the chicks are fed with their respective feed and care is executed for the incorporation of any other edible stuff. The present study experiment is in accordance with who demonstrated an experiment in which a complete of 300, 7 days older chicks were arbitrarily allocated to three sets. He also stated that the chickens stayed nourished by corn soy containing appetizer foods (controller set) and accompanied by 1.5 and 3% lysine (tentative sets) throughout fifteen days, as a result greater body masses stayed witnessed within sets.

In the present study, maximum amount of lysine curbed in available literature is 3 g/kg of feed and safe levels of lysine in sustenance of chicks are conserved. This parameter is in accordance with many researches like who described the evaluating of L-lysine constraint of developing chicks using 13 g lysine kg^{-1}; plus who described the idyllic amino acid outline meant for chick; similarly manifested the lysine requirements of preliminary chicks plus consequent effects throughout the developing stage 1, 2; and also determined the bio-availability of lysine commencing a fluid lysine cradle in chicks.

In the present study, the weights (mean) of chicks before and after the completion of each trial are described respectively. Growth parameters are perceived and deliberate the weight gain as well. Difference in the body weights of experimental sets is also calculated and inscribed. Also, any sort of behavioral changes during study is logged and observe thoroughly.

The experimental results indicated that the L-lysine produced in a crude form through fermentation using bacterial strain has sufficient enough to increase the biomass of chicks. The quantity of meat among the growing chicks has increased by adding the formed product in the feed. The crude fermentation product produced by bacterium has shown no contamination and seeds of the plant remain fresh for 15 days trial. On the other hand, feed produced by adding standard lysine mono-hydrochloride results in the growth of shots and roots in seeds, indicating that the solution of lysine mono-hydrochloride is also suitable to break seed dormancy plus increasing rooting and shooting capabilities of pearl millet.

According to the cost estimation of the present study, the price of the product formed in the experiment is Rs.43.2 per kg while the price of available commercial feed is Rs.50.0 per kg. The difference of price between the product and commercial feed is Rs.6.8. The cost of production for the experimental feed is much less than the cost of available commercial feed. The results obtained by experimental feed are tremendous, opening a new era in the production of feed for poultry industry. Another benefit of the experimental product is that it is free of any sort of contamination and haram stuff while the tremendous results indicating that the product efficiency is much higher than the results of commercial feed.

CONCLUSION

In the present study L-lysine was produced exclusively by *Bacillus subtilus*, *Streptococcus sp.*, and *Bacillus sp.* obtained from nature. While good production strains have been obtained, the characters required for high productivity still remain largely unknown. Much effort has recently been devoted to elucidate the mechanisms of microbial production of L-lysine. The biosynthetic pathways of L-lysine are now well known, and the focus of attention has therefore been moved to metabolic control and its breakdown. In the normal metabolism of microorganisms, L-lysine synthesis is in equilibrium with requirements, *i.e.,* it is carried out under limiting conditions. The accumulation of large quantities of L-lysine is therefore a pathological phenomenon arising from an artificial distortion of metabolism in the strain. In this respect, L-lysine production is essentially different from traditional catabolic fermentations. As a result, more research on appropriate fermentation technology is needed.

In the present study L-lysine was produced in a liquid form as the end product and was not pure, comprised of different other fermentation products like glutamic acid etc. Hence, it is an ample need of the hour to find out the purification approaches for L-lysine. Also, various methods are required to change the form of the end product from liquid to dry granular powder form.

In the present study the application of L-lysine produced was checked by performing a simple experiment on chicks. Growth parameters were perceived and deliberated the weight gain as well. However, more parameters are still needed to check the efficiency of the product. The experiment design in the present study was comprised of limited amount of chicks; hence, large amounts of chicks are needed to plaid the experiment at industrial levels.

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