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A biotechnological process for treatment and recycling of cage layer manure as a feed ingredient for egg type chicken

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ABSTRACT

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A study was undertaken to evaluate the nutritive and feeding values of fermented and unprocessed cage layer manure (CLM) as a feed ingredient for egg type chicken. Ten samples of CLM were collected from different farms and analyzed. The mean crude protein, true protein, uric acid, calcium and phosphorus in unprocessed CLM were 17.42, 12.27, 6.30, 7.78 and 1.61 per cent. The neutral detergent fibre, acid detergent fibre, hemicellulose and cellulose content of CLM were 46.03, 30.13, 15.90 and 17.79 per cent. In comparison to unprocessed CLM, fermented CLM had low crude protein (16.86%), true protein (8.19 %) and uric acid (5.76%). The AME of unprocessed and fermented CLM were 1050 and 1013 kcal/kg. The *E.coli, Salmonella, Staphylococci, Clostridium* and yeast count in unprocessed CLM were 6.9, 4.8, 9.5, 9.8 and 6.9 $\log_{10}$ cfu/g. In fermented product, *E.coli, Salmonella, Clostridium* were absent and *Staphylococci* was reduced from 9.7 to 5.3 $\log_{10}$ cfu/g. *Lactobacillus* count in the fermented product was 12.36 $\log_{10}$ cfu/g. Feeding trial was conducted in egg type chicken to find out maximum level of inclusion of fermented and unprocessed CLM, chicks were divided into seven groups (with three replicates in each group) and fed with isocaloric and isonitrogenous diet containing 0, 5, 7.5, 10 per cent fermented CLM and 5, 7.5 and 10 per cent unprocessed CLM. During 0 to 4 weeks, the weight gain, feed intake and feed efficiency of the fermented CLM was comparable to control except for reduced feed intake in 7.5 per cent fermented CLM fed groups. The weight gain, feed intake and feed efficiency were not influenced by use of fermented and unprocessed CLM up to 10 per cent during 5 to 8 weeks of age or during the chick phase (0 to 8 weeks of age).

Feeding of fermented and unprocessed CLM did not influence weight of organs (gizzard, heart, intestine) expressed in percentage of live weight, intestinal length and pH. Serum uric acid, serum protein, serum albumin and globulin were not influenced by feeding different levels of unprocessed and fermented CLM. Microbial load of jejunum showed increased *E.coli, Salmonella, Staphylococci* and *Lactobacillus* count in treated groups than control. *Clostridium* was absent in all groups. Microbial population of excreta from birds fed with fermented and unprocessed CLM showed comparable *E.coli, Lactobacillus* in all treated groups and higher *Salmonella, Staphylococci* and *Clostridium* count in unprocessed CLM fed groups. Villi length, epithelial thickening, crypts number per field and width were not influenced by feeding different levels of fermented and unprocessed CLM. Significant increase in villi width was noticed in the birds fed fermented CLM. The study reveals that the problem of health hazard in poultry due to feeding of CLM as feed ingredient was overcome by feeding (upto 10% level) CLM fermented with lactobacillus.

Key words: Cage layer manure, fermentation, chemical composition, microbial load, feeding value, egg type chicken

INTRODUCTION

Cage layer manure (CLM), a biological waste whose production has increased in proportion to the growth of the layer population, is locally available in the layer farms, easy to collect large amount in a small area, transportation cost is negligible (as source and user are in same premises), they are not competitive to human food and reduce environmental pollution. Exploitation of its (CLM) usage in a form acceptable by the feed handler and poultry shall maximize the usage and cut on the feed cost.

Layer populations in India was around 185 million in 2003, producing roughly 2.5 million tonnes of dried CLM. Currently, these wastes are purchased for application as fertilizer, biogas and electricity generation. Use of CLM as a fertilizer causes environmental problems like nitrate leaching (Unwin *et al*., 1991) and in electricity generation, the high ash content (approximately 35 per cent) in CLM causes potencial slagging fuel problem (Kelvin *et al*., 2002). Hence an alternate way of disposing these wastes needs to be evaluated. One of the alternate approach will be recycling by feeding. The crude protein (31.08%), calcium (8.27%) and phosphorus (2.00%) content of CLM make the waste a good source of non-conventional feed (Biely *et al*., 1972). But the presence of pathogenic microorganism in the poultry manure may cause some health hazard for livestock in general and poultry in particular. (Shih, 1993).

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Problem such as loss of organic nutrients, risk in handling concentrated chemicals are encountered in physical / chemical method of processing. So it is imperative to develop an efficient process to treat the organic wastes and convert them to useful and safe resources. Making the conversion process efficient and economical, the modern biotechnological process like fermentation is used. Use of microbial controlled fermentation by acid producing microorganism like *Lactobacillus* convert the waste into useful and safe resources (Wooley *et al*., 1981; El Jalil *et al*., 2001). The propagation of *Lactobacillus* in field is not only a simple process but also cheaper. Hence the present study was undertaken to ferment Cage Layer Manure with Lactobacillus and to assess the performance of egg type chicken fed fermented CLM.

MATERIALS AND METHODS

Ten samples of CLM collected from different farms and all the experimental chick mashes formulated for growth trial were analyzed for proximate principles, calcium and phosphorus content as per AOAC (1995), true protein (Sastry *et al.,* 1999) and fibre fractions (Goering and Van Soest, 1970). Uric acid was extracted from CLM as per AOAC (1995) and estimated by phosphotungstate method (Caraway, 1955). pH of cage layer manure were analyzed by homogenizing 25g of sample with 225 ml of distilled water in a blender for 2 minutes. The homogenate was filtered through muslin cloth and the filtrate was used for determining pH.

Five samples of unprocessed CLM, five samples of fermented CLM and excreta of birds fed different experimental chick mash in feeding trial were evaluated for microbial count. The samples were diluted as follows: One gram of the material (unprocessed or fermented CLM) was placed in the test tube containing 9 ml of sterilized physiological saline solution (Nacl, 8.5 g/L) and thoroughly mixed. This suspension was serially diluted up to 10^{-9} and 0.1 ml of appropriate dilutions were inoculated on the selective agar plates by spread plate method. The plates were incubated at 37°C for 24 hours. Deoxycholate Lactose agar, Salmonella Shigella agar, Baird Parker agar, Clostridial agar, Sabouraud Dextrose agar and MRS agar were used as the medium for *E.coli*, *Salmonella, Staphylococci, Clostridium*, yeast and *Lactobacillus* count respectively. After incubation, the colonies were counted as the numbers of colony forming units per gram of sample and expressed in $\log_{10}$ (cfu/g) (Quinn *et al*., 1992).

Biological processing of cage layer manure

Lactobacillus acidophilus used for fermentation was isolated from commercial probiotics (Provilacc-containing *Lactobacillus acidophilus*-2340 million, Manufactured by Tetragon Chemie Pvt Ltd, Bangalore) available in the market and it was morphologically confirmed by Gram staining, growth at 15°C and 45°C in MRS agar, catalase test, gas producing property from glucose and motility test (Barrow and Feltham, 1993) .

Fermentation of CLM was done as described by El Jalil *et al.* (2001). The collected CLM was sun dried and pulverized. The ground CLM was diluted with tap water in the proportion of 1:1 and mixed with molasses at 10 per cent level. The pH was adjusted to 6.5 using 50 per cent sulfuric acid solution. *Lactobacillus acidophilus* was inoculated at different concentrations *viz* 8 x 10^9, 6 x 10^9 and 4 x 10^9 cfu/g in poultry manure. The inoculated mixture was packed airtight in polythene bags to study the effect of pH change at different time intervals (0, 4, 8, 12, 16 and 20 days after incubation). For the biological experiment, *Lactobacillus acidophilus* was inoculated and incubated for 12 days and the level of *Lactobacillus acidophilus* was maintained at 4 x 10^9 cfu/g of the finished chick mash as recommended by Haddadin *et al.* (1996).

Feeding trial

The metabolizable energy of unprocessed and fermented CLM was estimated using fourteen adult cockerels (21 weeks old) as per the method of Sibbald (1976).

Seven different experimental chick mashes were formulated with 0, 5, 7.5 and 10 per cent fermented CLM and 5, 7.5 and 10 per cent unprocessed CLM. All the diets were formulated to have same levels of protein, metabolizable energy, calcium and phosphorus.

Day-old egg type chicks (252 numbers) were weighed individually, wing banded and distributed randomly into seven groups with three replicates (12 chicks in each replicate) in each group. The chicks were reared in cages. They had free access to feed and water. Chick mash was fed from day-old to 8 weeks of age. Standard managemental practices were followed uniformly for all the groups except the feed. Weekly body weight and feed intake were recorded.

At the end of 8 weeks, six birds per treatment were selected to study the digestibility of dry matter of the experimental chick mash. The birds were starved for 48 hours and were fed individually, feed was offered for the period of 5 hours with each of seven experimental rations and excreta was collected after 24 hours of feed withdrawal. The dry matter content of the chick mash and the excreta were quantified and the dry matter digestibility was calculated.

At the end of eight weeks of age, six birds from each treatment were selected randomly and slaughtered for recording the weight of liver, heart and gizzard. The length and weight of intestine were also recorded. The pH of jejunum

content was estimated by using pH meter. Blood was collected for analyzing the serum uric acid by phosphotungstate method (Caraway, 1955) and serum protein by the modified Biuret and Dumas method (Varley, 1980).

The intestinal contents were collected aseptically for microbial count namely *E. coli*, *Salmonella*, *Staphylococci*, *Clostridium* and *Lactobacillus* count as per the standard procedure.

The jejunum was collected and preserved in 10 per cent neutral buffered formalin for histological studies. The tissue was molded in paraffin and the paraffin embedded tissues were sectioned to 4-µ thickness, stained with haematoxylin and eosin for histomorphological and histopathological examination (Bancroft and Stevens, 1996).

By using the ocular micrometer the length and width of the intestinal villi, epithelial thickening, crypts number per field and width were measured and recorded.

Statistical analyses

The data collected from various parameters were statistically analyzed as per the method of Snedecor and Cochran (1989) and Duncan (1955). The data in percentage were subjected to arc sine transformation (Gomez and Gomez, 1984) and analyzed.

RESULTS AND DISCUSSION

Proximate composition, true protein, uric acid, calcium, phosphorus and fibre fractions of unprocessed cage layer manure (CLM) and fermented CLM are presented in Table 1. In comparison to unprocessed CLM, fermented CLM had low crude protein, true protein and uric acid which was in agreement with the report of Ahuja *et al.* (1983) and El Jalil *et al*. (2001). This change has been attributed to the transformation of protein nitrogen into non-protein nitrogen by some microorganisms present in poultry manure / L*actobacillus* used for fermentation or endogenous enzymes from the bird. The fermented CLM prepared in this study was free from objectable odour. The elimination of odour observed was in agreement with the reports of El Jalil *et al.* (2001). The low level of ammonia due to fermentation of CLM with *lactobacillus* has been attributed to deodorization (El Jalil *et al*., 2001). The marginal low level of crude fibre in fermented CLM observed was in agreement with the study of El Jalil *et al*. (2001) and the effect has been attributed to the action of cellulolytic bacteria in poultry manure during fermentation.

The pH of test material before fermentation was 7.86. After *Lactobacillus* inoculation concentration (cfu/g) of 8 x 10^9, pH was lowest on day 8 (Table 2). Where as, concentration of 6 x 10^9 and 4 x 10^9, pH was lowest on day 12. Further incubation, resulted in raise of pH. However, El Jalil *et al*. (2001) reported incubation of *lactobacillus* in CLM (ash-18.6 to 22.8%) resulted in pH reduction to the extend of 4.0 to 4.2. The unsuccessful attempt to achieve pH of 4.0 in this experiment might be due to high ash content (34.14%) which are known to have high buffering capacity as reported by Ashbell *et al.* (1995).

The test material for fermentation was selected based on the sample having highest count of *E.coli* and *Salmonella*. The test sample fermented with *Lactobacillus* at different concentration (8 x 10^9, 6 x 10^9 and 4 x 10^9 cfu/g) was found to

Table 1. Chemical composition of unprocessed and fermented cage layer manure

Parameter	Cage layer manure	
	Unprocessed	Fermented
Proximate composition		
Dry matter	94.56±0.35	92.98±0.10
Protein	17.42±0.75	16.86±0.68
Ether extract	0.71±0.07	1.10±0.04
Crude fibre	14.51±0.42	13.27±0.25
Total ash	34.14±1.43	36.50±0.64
Nitrogen free extract	33.09±1.30	32.26±0.91
Acid insoluble ash	12.34±1.42	13.37±1.12
Nitrogen fractions		
True protein	12.27±0.64	8.19±0.76
Uric acid	6.30±0.55	5.76±0.72
Minerals		
Calcium	7.78±0.63	6.28±0.34
Phosphorus	1.61±0.17	1.94±0.02
Energy		
AME (kcal/kg)	1050	1013
TME (kcal/kg)	1455	1447
Gross energy (kcal/kg)	2430	2430
Metabolizability of GE	0.432	0.417
Fibre fractions		
Neutral detergent fibre	46.03±0.84	46.83±2.49
Acid detergent fibre	30.13±1.10	32.02±0.57
Hemicellulose	15.90±0.84	14.81±1.95
Cellulose	17.79±1.34	18.43±0.26

Table 2. Changes in pH during fermentation period

Fermentation period (days)	Lactobacillus inoculum @ 8x10^9 cfu/g	Lactobacillus inoculum @ 6x10^9 cfu/g	Lactobacillus inoculum @ 4x10^9 cfu/g
4th day	5.47±0.15	5.42±0.08	5.55±0.17
8th day	5.01±0.03	5.15±0.05	5.34±0.07
12th day	5.13±0.05	5.11±0.06	5.18±0.05
16th day	5.12±0.03	5.25±0.02	5.25±0.05
20th day	5.21±0.02	5.36±0.01	5.34±0.06

The mean pH of five unprocessed cage layer manure was 7.74±0.15
The pH of the test material was 7.86 and it was adjusted to 6.5 using 50 per cent sulfuric acid solution in the initial mixture
Each value is the mean ± SE of three observations

Table 3. Microbial Profile of unprocessed and fermented cage layer manure (in $\log_{10}$ cfu/g)

Micro-organism	Unprocessed cage layer manure*	Cage layer manure taken for fermentation	After fermentation*
E. coli	6.9±0.88 ($9.1x10^6$)	8.5 ($3x10^8$)	Absent
Salmonella	4.8±1.04 ($6.0x10^4$)	8.6 ($4x10^8$)	5.8** ($6.0x10^5$)
Staphylococci	9.5±0.32 ($2.8x10^9$)	9.7 ($4.8x10^9$)	5.3±0.33 ($2.1x10^5$)
Clostridium	4.8±1.54 ($6.7x10^4$)	3.0 ($1x10^3$)	2.5** ($3.0x10^2$)
Yeast	6.9±0.80 ($7.2x10^6$)	8.0 ($1x10^8$)	9.8±0.13 ($6.6x10^9$)

*Each value is the Mean ± SE of five observations
**Of five fermented samples assayed only one sample had *Salmonella* and *Clostridium* count
The value in the parenthesis is the mean of the microbial count in cfu/g

eliminate 100 per cent *E.coli*, *Salmonella* and *Clostridium* (except in one where in 37 and 33 per cent of *Salmonella* and *Clostridium* were eliminated) (Table 3). The destruction of *Enterobacteria* in the fermented CLM was considered as the most important factor for the product safety. Fermentation was found to eliminate 44 per cent of *Staphylococci* and resulted in product with *Lactobacillus* count of 2.3×10^{12} cfu/g. Similar results were also observed by El Jalil *et al*. (2001). The reduction in microbial load may be due to the rapid establishment of anaerobic condition and development of lactic acid (by *Lactobacillus*) suppressing the activity of undesirable microorganism as occurring in the intestinal tract of birds fed probiotics (Heres *et al*., 2003).

Metabolizable energy of cage layer manure

The apparent metabolizable energy of CLM (1050 kcal/kg) was within the range of the earlier report (Nesheim, 1972; Ichhponani and Lodhi, 1976; Ramteke *et al.,* 1994; Maqbool Ahmed *et al*., 1996). The true metabolizable energy of CLM was 1455 kcal/kg. The ratio of AME and TME (0.72) was very low, suggesting that low digestibility of CLM would have resulted in more loss of endogenous component. The mean gross energy content of CLM was 2430 kcal/kg, which was within the range of 2052 to 2570 kcal/kg reported by Chandrasekaran (1993). The low gross energy of CLM might be due to high ash content and low metabolizability might be due to high neutral detergent fibre in CLM than other cereals.

Feeding trial

The response of feeding different levels of fermented and unprocessed CLM in egg type chicken on weight gain, feed intake and feed efficiency are presented in Table 4. During 0-4

Table 4. Response of feeding different levels of fermented and unprocessed cage layer manure on production performance in egg-type chicken

Treatment	Weight gain* (g)			Feed Intake** (g)			Feed efficiency**		
	0-4 Week	5-8 Week	0-8 Week	0-4 Week	5-8 Week	0-8 Week	0-4 Week	5-8 Week	0-8 Week
Control	201.1±3.9^a	294.4±5.7	495.5±7.8	433±1.5^a	951±2.2	1383±2.9	2.15±0.04	3.23±0.06	2.79±0.03
5% fermented CLM	198.3±3.8^a	288.3±6.0	486.6±8.7	4432.1^a	997±18.6	1440±20.7	2.24±0.11	3.47±0.17	2.97±0.14
7.5% fermented CLM	198.0±4.2^a	270.3±6.9	470.6±9.3	407±1.2^b	993±38.2	1396±41.0	2.06±0.05	3.66±0.17	2.96±0.12
10% fermented CLM	190.8±3.7^{ab}	284.5±6.7	475.3±7.7	431±11.1^a	959±7.4	1390±7.9	2.26±0.07	3.38±0.15	2.93±0.08
5% unprocessed CLM	191.3±3.4^{ab}	276.2±5.5	467.2±6.7	405±11.9^b	978±50.8	1383±60.3	2.12±0.08	3.55±0.04	2.96±0.06
7.5% unprocessed CLM	185.4±4.4^{bc}	278.8±7.0	464.9±9.9	434±7.7^a	990±36.6	1425±44.2	2.35±0.10	3.58±0.19	3.08±0.15
10% unprocessed CLM	175.6±3.3^c	294.2±6.0	470.2±7.5	387±6.7^b	966±11.2	1353±17.3	2.20±0.06	3.29±0.07	2.88±0.05
Critical difference	P< 0.05=10.64	NS	NS	P<0.05=22.2	NS	NS	NS	NS	NS

*Each value is the mean ± SE of thirty-six observations
**Each value is the mean ± SE of three observations
(Means with at least one common superscript in a column do not differ significantly at P< 0.05)

Table 5. Gut micro flora of egg-type chicken fed with rations containing fermented and unprocessed cage layer manure (log_{10} cfu/g)

Treatment	*E.coli*	*Salmonella*	*Clostridium*	*Staphylococci*	*Lactobacillus*
Control	3.9	2.8	Absent	2.0	4.4
5% fermented CLM	3.1	3.1	Absent	2.6	4.9
7.5% fermented CLM	3.7	3.9	Absent	3.0	5.7
10% fermented CLM	4.7	3.5	Absent	3.5	4.7
5% unprocessed CLM	2.7	3.3	Absent	2.7	4.2
7.5% unprocessed CLM	3.7	4.2	Absent	3.4	4.4
10% unprocessed CLM	5.2	4.0	Absent	3.1	4.5

weeks of age, inclusion of fermented CLM up to 10 per cent level and unprocessed CLM up to 5 per cent level, comparable weight gain was observed. Feeding of unprocessed CLM at 7.5 and 10 per cent level resulted in significant lower weight gain. Fermented CLM included groups did not influence feed intake (0 to 4 weeks) whereas unprocessed CLM fed groups had lower feed intake. The different levels of fermented and unprocessed CLM inclusion did not influence feed efficiency in 0-4 weeks of age. The weight gain, feed intake and feed efficiency were not influenced when fermented CLM and unprocessed CLM were fed between 5 and 8 weeks of age or during the chick phase of 0-8 weeks of age. Similarly in egg type chicken, Biely *et al*. (1972) reported unprocessed CLM can be incorporated up to 15 per cent with out any deleterious effect on weight gain, feed intake, feed efficiency and Ahuja *et al*. (1974) also reported weight gain was comparable up to 20 per cent except for low feed efficiency at inclusion level of 10 per cent and above. Similar observation of comparable weight gain, feed intake was noticed (El Jalil *et al*., 2001) using fermented cage layer manure up to 20 per cent in layer birds and (Mandal *et al.*, 1996) fermented quail manure up to 20 per cent in quail mash. The comparable weight gain, feed intake and feed efficiency with CLM (fermented and unprocessed) suggest that the same could be used in egg type chick mash up to 10 per cent level and the presence of uric acid (6.30±0.55) and microbial population did not affect the parameters studied.

The dry matter digestibilities of different experimental ration were not significantly different. The comparable dry matter digestibility of all experimental ration implies that the nutrients digestibility of the experimental ration might not be affected by the incorporation of CLM up to 10 per cent levels

Serum uric acid and serum protein, albumin and globulin level were found to be unaffected by different levels of feeding fermented and unprocessed CLM.

Feeding fermented and unprocessed CLM at different levels did not influence the dressing percentage and proportional weight of the organs- gizzard and heart. Feeding of unprocessed and fermented CLM up to 10 per cent level from day one to 8 weeks did not influence the intestinal weight, length and pH.

Gut microflora of egg type chicken

E.coli, *Salmonella, Clostridium, Staphylococci* and *Lactobacillus* count in the intestinal content of egg type chicken fed with different levels of fermented and unprocessed CLM are presented in Table 5. *E.coli* count of the intestine was found to increase as the level of CLM (fermented and unprocessed) was increased. Fermentation was found to have little effect in reducing the *E.coli* count. At 5 and 7.5 per cent inclusion of fermented and unprocessed CLM the *E.coli* was less than control. The *Salmonella, Staphylococci* and *Lactobacillus* count of birds fed with cage layer manure (fermented and unprocessed) was higher than control and fermentation had little effect in reducing *Salmonella* and *Staphylococci* count or increasing *Lactobacillus* count. *Clostridium* was absent in all the seven experimental diets fed birds. This was concurred with the result of Jin *et al.* (1998) that the incorporation of probiotics (containing Lactobacillus) did not influence *Lactobacillus*, total anaerobes, total aerobes and *Streptococci* count in the intestine. In *invitro* study *Lactobacillus* fermented CLM was free from *E.coli*, *Salmonella* and *Clostridium* and *Staphylococci* was reduced by 44 per cent (Table 3). In spite of the beneficial result observed in the fermented product, the microbial profile of the intestine of bird fed fermented and unprocessed CLM was found comparable. However, use of *Lactobacillus* as probiotics in feed and water had been established to reduce the intestinal pathogens (Fuller, 1973).

Feeding of CLM fermented by *Lactobacillus* did not show any significant difference in villi length, epithelial thickening, crypts number per field and width when compared to control and unprocessed CLM. Microscopical examination of the intestinal sections of birds fed with different experimental ration did not revealed any characteristic alternations except for the mild infiltration of mononuclear cells in lamina properia.

Presence of pathogenic micro organism in poultry manure may cause some health hazard in poultry when we used cage layer manure as a feed ingredient. But we overcome this problem by fermenting the CLM with *Lactobacillus*. Fermentation of CLM was beneficial in deodorizing and reducing the pathogens and make the product safe for the handler and to the poultry. Thus the present study would partially / totally solve the problem of CLM disposal and reduce environmental pollution.

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