

Comparative Effects of Coated Compound and Mono-component Proteases on Growth Performance and Nutritional Efficiency in Broiler Diets

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Abstract: Protease as feed additive is being used in poultry production as a partial replacement for protein sources for cost efficiency and reducing nitrogen excretion. However, diverse proteases may yield different responses under field conditions. A pellet diet study was conducted in Cobb broilers to assess the impact of coated compound (CC) and mono-component (MC) proteases with 5% replacement of digestible amino acids and 0.9% crude protein. Birds fed positive control diet had a better growth than those fed negative control diet, regardless of enzyme supplementation. However, CC protease had shown feed conversion ratio (FCR) like control in a reformulated diet, whereas negative control and MC protease missed to gain the feed conversion. In measures of nutritional efficiency, like energy efficiency, protein efficiency and amino acids efficiency (lysine and methionine), the CC protease proved to be better than MC protease. In terms of European efficiency factor (EEF), control and CC protease elicited a closer response, whereas the other two groups showed a drop. In this study, CC protease allowed partial substitution of digestible amino acids and crude protein, while maintaining feed efficiency and animal performance. It could be concluded that incorporating CC proteases is an efficient choice to maximize the utilization feed material resources and efficiency in animal protein production.

Key words: Amino acid, broilers, compound protease.

1. Introduction

Soybean meal is one of the most commonly used protein sources for poultry. Most of the proteins in soybean are easily digested, except some, like glycinin, protease inhibitors and antigenic proteins which may predispose the intestinal damage and impair the immune function [1]. With the development of enzyme technologies, protease application helps to enhance the protein and amino acid digestibility [2], as well encourages the addition of alternate protein feed materials which helps to reduce the feed cost. However, different proteases with different inherent characteristics may elicit divergent responses *in vivo* [3].

In the present study, to enrich proteases, a

compound protease was developed with acid, neutral and alkaline proteases produced by *Aspergillus niger*, *Bacillus subtilis* and *Bacillus licheniformis*, respectively.

The coated compound (CC) protease enzyme products were mixture of acidic, neutral and alkaline proteases coated by pH sensitive polymers, which could successively dissolve and work in different microenvironment of digestive tract. It was hypothesized that the CC proteases could improve the utilization of protein and amino acids in different intestinal segments, and thus improve the animal productivity, nutritional efficiency and cost efficiency in broiler production. Therefore, the objective of this study was to evaluate the comparative efficiency of CC protease and mono-component (MC) protease on broiler production and economic parameters.

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2. Materials and Methods

2.1 CC and MC Proteases

The proteases used in the study were commercially available for the use in animal feeds. The CC proteases were in granular form provided by Kemin Industries South Asia Private Limited, containing acidic, neutral and alkaline proteases produced by *Aspergillus niger*, *Bacillus subtilis* and *Bacillus licheniformis*, respectively. All the types of proteases were coated with different polymers to provide thermostability at pelleting conditions and for targeted release in the gastro intestinal tract. The acidic protease was coated with heat resisting membrane, and the neutral and alkaline proteases were both coated by heat-proof and acidity-resisting coating layers, which were not expected to degrade by the gastric acid. Therefore, CC protease could successively dissolve and work in acidic, neutral or alkaline environment in the digestive tract.

The MC protease sourced commercially expresses the activity of alkaline protease and is mentioned as a fermentation product from *Bacillus* spp..

2.2 Animals and Experimental Designs

Eight hundred one-day-old Cobb broiler chicks were randomly allotted into four dietary treatments, each with 10 replicates of 20 birds, equal male and female each in an open deep litter for 42 d. The groups included a positive control with basal diet, negative control (Table 1) with 5% relative reduction in digestible amino acids and approximately 0.9% crude protein, and treatments were supplemented with MC protease and CC protease, respectively, as per the commercial recommendations.

The feed and water was fed *ad libitum* and farm medication and rearing practices followed were same in all the groups as per commercial management practices.

2.3 Diets

Positive control diet had a crude protein content of 22.6%, 21.5% and 19.6% and digestible lysine of 1.27%, 1.20% and 1.01% for pre-starter, starter and finisher period, respectively. Negative control had relatively 5% lesser digestible amino acids and 0.9% lesser crude protein than positive control. The diet was having corn, soybean meal, mustard seed meal and meat cum bone meal as base ingredients. Reformulation was allowed to substitute relatively expensive soybean meal, synthetic amino acids with corn and other inexpensive protein meals.

2.4 Data Collection and Statistical Analysis

The body weight and feed intake were measured on weekly basis for individual birds and cumulated for the replicate. Feed conversion ratio (FCR) was calculated with the average bird weight gain and the feed intake of the respective group (feed intake as g feed/g gain). European efficiency factor (EEF) and nutritional efficiency for energy, protein, lysine methionine, methionine and cysteine have been calculated at the end of the trial. EEF was calculated according to Mohammadi et al. [4] by Eq. (1):

$$EEF = \frac{\text{livability (\%)} \times \text{body weight (kg)} \times 100}{\text{age of capitalization in days} \times \text{FCR}} \quad (1)$$

The energy efficiency has been assessed by body weight gain per 100 kcal energy intake and for other nutrients by body weight gain per gram of nutrients intake [5]. As a part of productive and economic efficiency, total live body weight production (kg) per metric tonne of feed has been assessed from FCR (1,000 kg of Feed/FCR).

Mean values were calculated for each treatment group. One-way analysis of variance (ANOVA) was performed using Statgraphics Plus 5.1 software [6] to study the significance between different groups. The data were analyzed by least significant difference (LSD) method, and differences at $P < 0.1$ were considered significant.

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Table 1 Feed formulation and nutrient composition of experimental diets.

Ingredients (kg/1,000 kg)	Pre-starter (0-14 d)		Starter (15-28 d)		Finisher (29-42 d)	
	Positive control	Negative control	Positive control	Negative control	Positive control	Negative control
Corn (yellow)	551	581	575	599	626	657
Soybean meal	355	329	314	298	249	222
De-oiled rice bran	26	21	34	31	39	35
Meat cum bone meal	20	20	25	18	35	35
Calcite	11	11	10	11	9	9
Mustard de-oiled cake	10	10	20	20	25	25
Dicalcium phosphate	10	10	6	8	2	2
DL-methionine	3.12	2.99	2.67	2.37	2.37	2.27
L-lysine	2.82	3.05	2.93	2.83	2.10	2.56
L-threonine	0.60	0.72	0.58	0.43	0.76	1.08
Salt (common)	2.92	2.92	2.88	2.96	2.61	2.62
Soda bicarbonate	1.79	1.81	1.35	1.46	0.76	0.78
Additives*	6.00	6.00	6.00	6.00	6.00	6.00
Nutrients composition calculated						
Crude protein (%)	22.60	21.71	21.50	20.63	19.60	18.70
Crude fiber (%)	3.90	3.84	3.85	3.82	3.67	3.60
Ether extract (%)	5.12	4.77	6.00	5.76	6.76	6.43
Metabolizable energy (ME) (kcal/kg)	2,950	2,950	3,030	3,030	3,130	3,130
Calcium (%)	0.98	0.98	0.90	0.90	0.85	0.85
Available P (%)	0.50	0.50	0.45	0.45	0.42	0.42
Digestible lysine (%)	1.27	1.23	1.20	1.15	1.01	0.99
Digestible methionine (%)	0.60	0.58	0.55	0.51	0.49	0.48
Digestible cysteine	0.28	0.28	0.27	0.27	0.25	0.24
Digestible methionine & cysteine (%)	0.88	0.85	0.82	0.77	0.75	0.72
Digestible arginine	1.38	1.31	1.29	1.23	1.14	1.07
Digestible threonine (%)	0.75	0.73	0.71	0.67	0.66	0.66
Digestible tryptophan (%)	0.22	0.20	0.20	0.19	0.17	0.16
Chloride (%)	0.23	0.23	0.23	0.23	0.22	0.22
Sodium (%)	0.21	0.21	0.20	0.20	0.18	0.18

* Additives (kg): vitamin premix 0.5 kg, trace minerals 0.5 kg, mycotoxin binders 1.0 kg, organic acids 1.0 kg, nutritional emulsifiers 0.5 kg, choline chloride 0.5 kg, anticoccidials 0.3 kg, probiotics 0.5 kg, phytase 0.05 kg, non-starch polysaccharide degrading enzymes 0.1 and protease 0.3 kg.

3. Results and Discussion

The relative efficiency of MC protease and CC protease in commercial broilers and economical assessment has been shown in Table 2. The group supplemented with CC protease showed a lower feed intake than other groups ($P < 0.1$). No positive impact of protease in body weight gain was observed (Fig. 1). Supplementation of CC protease on diet reduced with amino acid and crude protein had shown a

significantly ($P < 0.1$) lower FCR (1.601) than negative control, but numerically better than positive control (1.603) and MC protease (1.626).

A similar kind of response on gain of feed intake and body weight has been observed on crude protein and amino acid reduced diets, as well as protease supplementation on reformulated diets [7]. The study also noted an increase in FCR in the negative control diets and enzyme added groups. Whereas, other

Table 2 Flock performance and economic efficiency of treatment groups from the *in vivo* assessment of CC and MC proteases in broiler diets.

Parameters	Positive control	Negative control	CC protease	MC protease	<i>P</i> value
Feed intake per bird (g)	3,585 ± 76.15 ^b	3,563 ± 59.90 ^b	3,489 ± 93.20 ^a	3,552 ± 80.86 ^b	0.0541
Final body weight (g)	2,277 ± 44.52 ^b	2,215 ± 66.87 ^a	2,220 ± 39.68 ^a	2,226 ± 51.76 ^a	0.0384
Body weight gain (g)	2,237.02 ± 44.68 ^b	2,174.40 ± 66.90 ^a	2,180.03 ± 39.60 ^a	2,185.37 ± 51.07 ^a	0.0381
FCR (g feed/g gain)	1.603 ± 0.036 ^{ab}	1.641 ± 0.072 ^b	1.601 ± 0.045 ^a	1.626 ± 0.046 ^{ab}	0.2651
EEF	336	323	332	325	NA
Livability (%)	97.50	98.50	98.50	98.00	NA
Live weight produced per tonne of feed (kg)	635.18	621.61	636.37	626.56	NA

^{a, b} Letters with in a row differ significantly ($P < 0.1$).

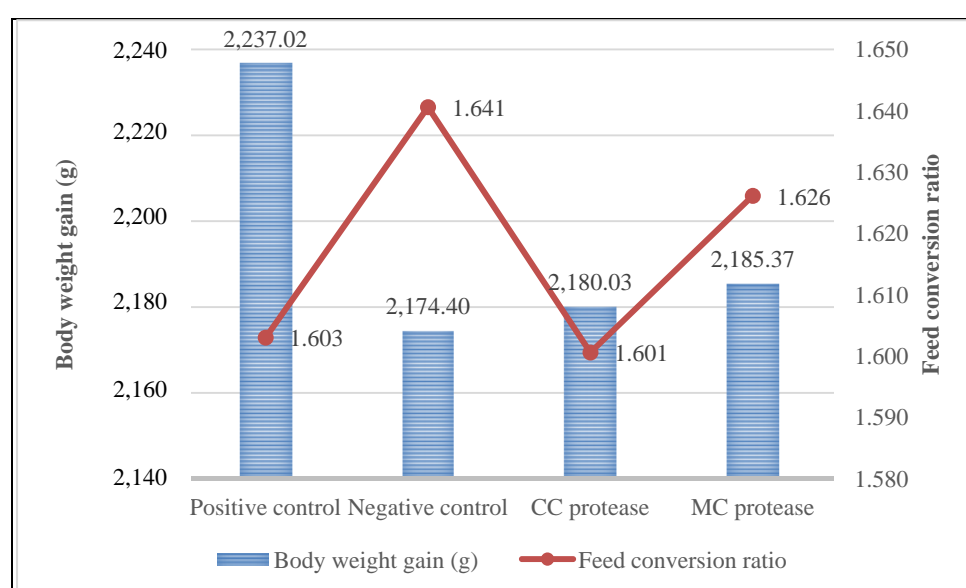


Fig. 1 Body weight gain (g) and FCR of broilers fed with CC protease and MC protease in amino acid reduced diets.

studies reported an increase in weight gain and reduced feed conversion with protease supplementation in crude protein and amino acid reduced diets [8, 9]. In contrast, another study mentioned the inconsistency in body weight gain and feed intake when proteases supplemented with crude protein and amino acid reduced diets [10]. However, in the present study, CC protease had shown significant improvement in feed intake than other treatment groups and FCR than negative control (Fig. 1). CC protease could revert the EEF equal to the basal diet in amino acids reduced formulations (Fig. 2 and Table 2).

Assessing the nutritional efficiency is a critical factor for animal protein production to understand

the nutrient utilization and optimizing the nutrients in the ration, as well as reducing the environmental pollution through undigested nutrients [1]. In the present study, addition of CC protease had shown significantly ($P < 0.1$) lesser energy and protein intake than other treatment groups [7] (Table 3). This might be due to the better nutrient utilization of CC protease than other groups [8].

It is also observed that protein efficiency ratio (Fig. 3 and Table 3) was significantly ($P < 0.1$) better for CC protease than basal diet, and numerically better than the other two dietary groups. A similar response was observed for amino acids, like lysine and methionine (Fig. 4 and Table 3). Similarly, increase in

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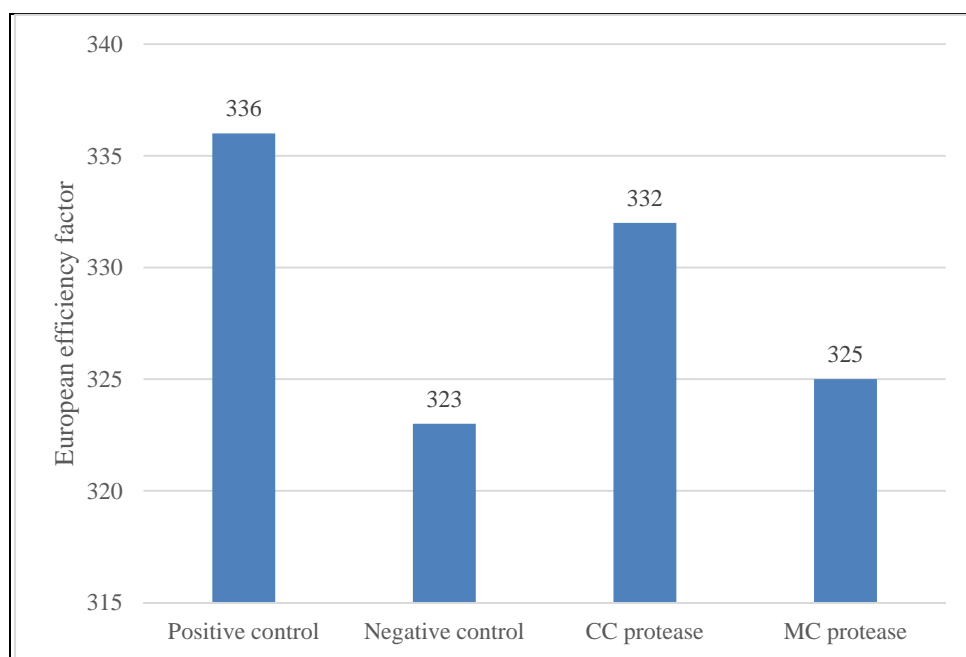


Fig. 2 EEF of broilers fed with CC Protease and MC protease in amino acid reduced diets.

Table 3 Nutritional efficiency of birds of treatment groups from the *in vivo* assessment of CC and MC proteases in broiler diets.

Parameters	Positive control	Negative control	CC protease	MC protease
ME intake per bird (kcal)	10,996.9 ± 235.91 ^b	10,931.0 ± 184.91 ^b	10,701.2 ± 285.68 ^a	10,897.9 ± 250.65 ^b
Protein intake per bird (g)	743.28 ± 15.39 ^c	706.80 ± 11.71 ^b	692.17 ± 18.60 ^a	704.62 ± 15.60 ^b
Lysine intake per bird (g)	40.06 ± 0.82 ^c	37.90 ± 0.63 ^b	37.12 ± 1.01 ^a	37.79 ± 0.82 ^b
Methionine intake per bird (g)	18.93 ± 0.39 ^c	17.61 ± 0.29 ^b	17.24 ± 0.46 ^a	17.55 ± 0.38 ^b
M + C Intake per bird (g)	28.49 ± 0.59 ^c	27.05 ± 0.45 ^b	26.49 ± 0.71 ^a	26.97 ± 0.60 ^b
EER (BWG/100 kcal ME)	20.35 ± 0.44 ^{ab}	19.90 ± 0.81 ^a	20.38 ± 0.60 ^b	20.06 ± 0.56 ^{ab}
PER (BWG/g of Protein)	3.01 ± 0.065 ^a	3.078 ± 0.12 ^{ab}	3.15 ± 0.09 ^b	3.10 ± 0.085 ^{bc}
LER (BWG/g of Lysine)	55.86 ± 1.22 ^a	57.40 ± 2.26 ^b	58.76 ± 1.71 ^c	57.85 ± 1.57 ^{bc}
MER (BWG/g of Methionine)	118.21 ± 2.56 ^a	123.55 ± 4.90 ^b	126.50 ± 3.65 ^c	124.57 ± 3.37 ^{bc}
MCER (BWG/g of M+C)	78.55 ± 1.70 ^a	80.42 ± 3.20 ^b	82.34 ± 2.38 ^c	81.07 ± 2.21 ^{bc}

^{a, b} Letters with in a row differ significantly ($P < 0.1$).

ME: metabolizable energy; EER: energy efficiency ratio; PER: protein efficiency ratio; LER: lysine efficiency ratio; MER: methionine efficiency ratio; MCER: methionine + cysteine efficiency ratio.

nitrogen retention was observed with the supplementation of protease in broiler chickens [11]. Whereas, in case of energy efficiency, CC protease had shown significant ($P < 0.1$) improvement over negative control, but numerical advantage over positive control and MC protease. A similar response of increase in apparent metabolizable energy (AME) from feed materials [12] has been observed with the supplementation of protease. This improvement might be due to the better amino acid utilization, which

contributes for certain level energy from amino acids and could create a better access to other endo and exogenous enzymes [2].

Better nutrient efficiency by protease supplementation might be due to the enhanced nutrient utilization, which minimizes the indigestible nutrients [8]. A similar response was observed for protein and amino acids when broilers fed with increased levels of digestible amino acids [5]. Whereas, the same study showed an increase in

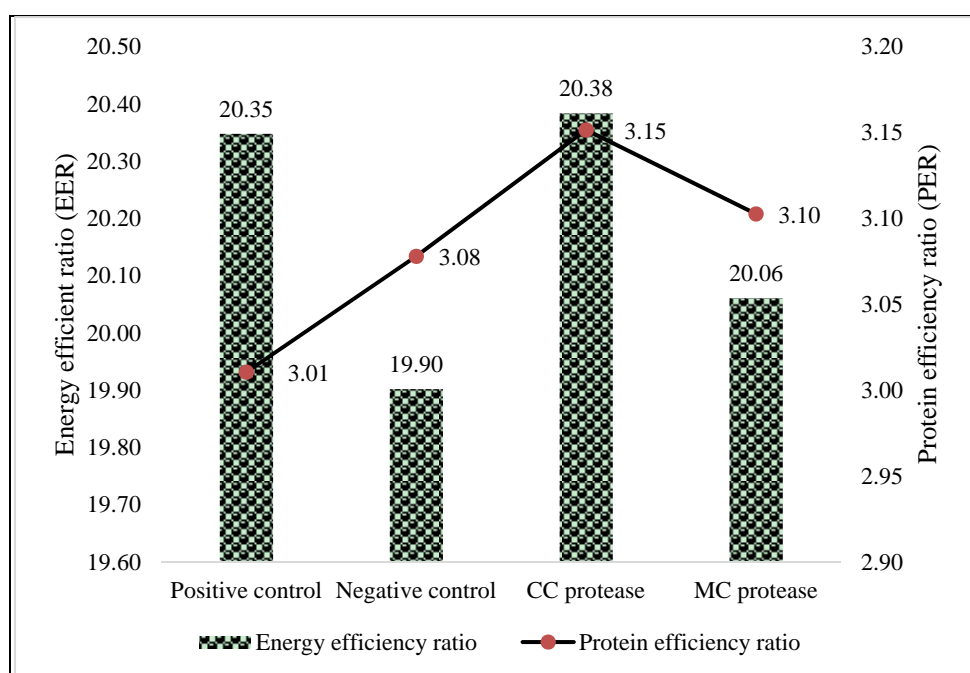


Fig. 3 Energy efficient ratio (EER) and protein efficiency ratio (PER) of broilers fed with CC protease and MC protease in amino acid reduced diets.

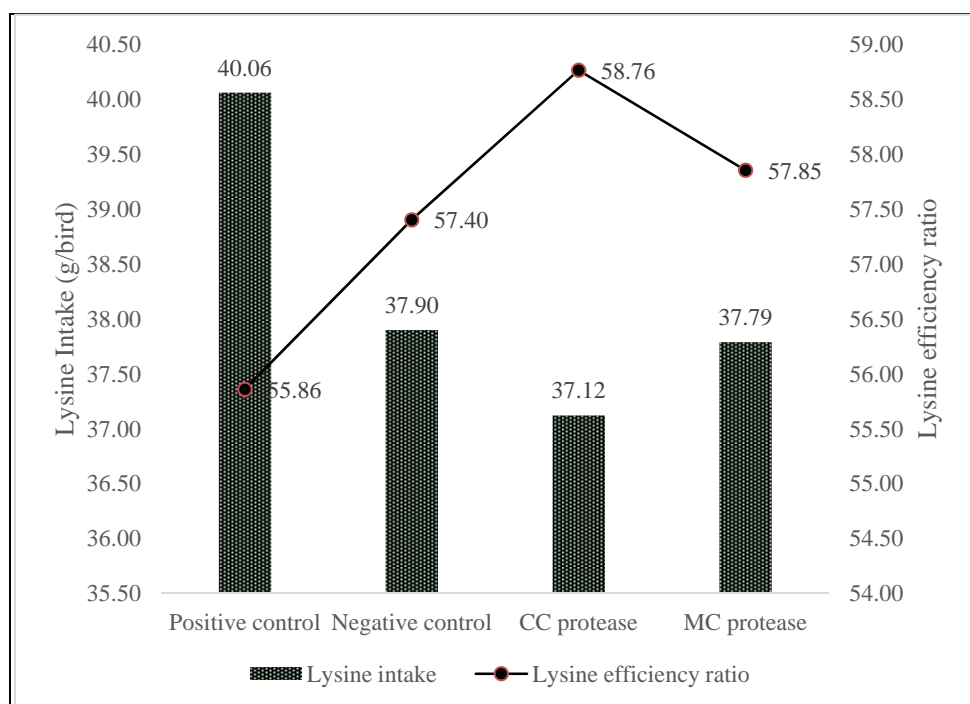


Fig. 4 Lysine efficiency ratio (LER) and lysine intake of broilers fed with CC protease and MC protease in amino acid reduced diets.

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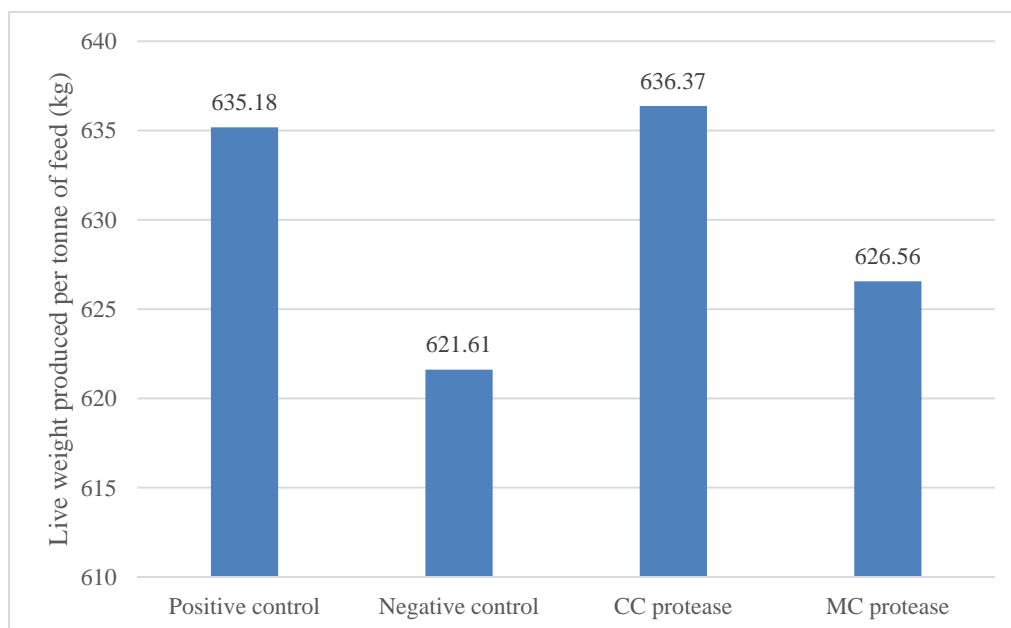


Fig. 5 Total live weight produced per metric tonne of feed from broilers fed with CC protease and MC protease in amino acid reduced diets.

energy intake and reduced energy efficiency in the diets supplemented with higher amino acid levels.

For live weight, the CC protease (636.37 kg) had shown a superior advantage over MC protease (626.56 kg) and negative control (621.61 kg) on the amount of live broiler produced per metric tonne of feed (Fig. 5).

4. Conclusions

Application of different proteases has improved the nutritional efficiency of broilers for better body weight gain, feed conversion and nutrient efficiency. CC protease has demonstrated a significant improvement in feed conversion, nutritional efficiency (energy, protein and amino acids) and economic efficiency than MC protease and other diets. It could be concluded that CC protease could be ideal option than MC protease for efficient and economical animal protein production. Further studies are also recommended on various diet density with strict quality control on feed raw materials to minimize the inconsistency from reformulated diets.

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