

NUTRITIONAL AND BIOLOGICAL METHODS OF NITROGEN EMISSION REDUCTION ON PRODUCTION PERFORMANCE OF GUINEA FOWL

A. Nithyavani¹, G. Srinivasan², A. V. Omprakash³, P. TensinghGnanaraj⁴,
B. Samuel Masilamoni Ronald⁵, R. Richard Churchill^{*6} and Pachipala Radha Satya⁷

Department of Poultry Science

Madras Veterinary College

Tamil Nadu Veterinary and Animal Sciences University

Chennai, Tamil Nadu – 600 007

ABSTRACT

*Reducing crude protein (CP) level in the feed without altering the amino acid contents by supplementing synthetic amino acids to reduce nitrogen excretion, adding of exogenous protease enzyme in feed to improve nitrogen utilization and treating litter with the culture of *Methylobacterium methanica* (MM) to prevent methane emission from litter are the proven methods in commercial poultry production, especially in broiler farming to protect the environment. However, the efficacy of these nitrogen emission reduction strategies on the performance and economics of guinea fowl in intensive production has not been studied adequately. Therefore, a biological experiment was conducted with high protein (HP) diets with (T1) or without (T2) fish meal (FM) and low protein (LP) diets added with exogenous protease enzyme with (T3) or without (T4) FM. The levels of critical amino acids in all the feed were balanced with the supplementation of synthetic amino acids. These treatments contained six replicates of each with 15-day-old keets. At the start of ninth week, the litter of three replicates from each treatment was sprayed with MM culture additionally, forming four more treatment groups, from T5 to T8 respectively. The results of the study indicated that replacing FM with plant protein sources did not affect body weight. The overall trend revealed that the reduction of CP by 2% had a negative ($P < 0.05$) impact on body weight; while, the addition of protease in feed had no notable effect. The feed consumption was not affected by the withdrawal of FM or reducing CP level by 2% or dietary protease supplementation or MM litter treatment. The feed efficiency was negatively impacted ($P < 0.05$) by withdrawal of FM in high and low protein diets;*

¹ Veterinary assistant Surgeon, Animal Husbandry Department, Government of Tamil Nadu, Vellore district

² Professor and Head, Department of Poultry Management, Suguna Institute of Poultry Management, Udumalpet, – 642 207

³ Professor and Head (Retd.), Poultry Research Station, Tamil Nadu Veterinary and Animal Sciences University, Madhavaram Milk Colony, Chennai – 600 051

⁴ Professor, Post Graduate Research Institute in Animal Sciences, Kattupakkam - 603203

⁵ Professor, Department of Veterinary Microbiology

⁶ Professor and Head, *Corresponding author, dechurchil@gmail.com

⁷ Postgraduate scholar

while, feed efficiency was not improved by protease supplementation in LP diets. The level of CP or withdrawal FM or protease enzyme supplementation in the ration did not affect livability. The MM litter treatment did not affect growth rate, feed consumption, feed efficiency and livability of guinea fowls. The results indicated that the benefit-cost ratio could be improved in guinea fowls by using low CP feed.

Keywords: Biological strategies, Growth performance, Guinea fowl, Nitrogen emission

Received : 27.05.2024

Revised : 152.08.2024

Accepted : 26.09.2024

INTRODUCTION

Guinea fowl, prevalent game birds in different regions of the world, have significantly contributed to the country's animal protein supply over time. Many people like the eggs and meat of guinea fowl due to their unique taste (Araújo *et al.*, 2023). In most developing countries, guinea fowl is raised under semi-intensive or extensive production systems with limited inputs (Issaka and Yeboah, 2016); whereas, in most European countries, guinea fowl production is carried out under modern intensive systems; where guinea fowl meat ranks second-largest source of poultry meat and eggs, following domestic chicken (Bernacki *et al.*, 2013).

Ammonia is produced by the microbial breakdown of uric acid in the poultry litter. The strategies to reduce ammonia emission from the litter include dietary manipulation with low crude protein (CP) diets supplemented with limiting amino acids, proper ventilation in poultry houses, the addition of chemicals like yucca saponin to the litter, antibiotics, zeolites, and phosphates and microbial treatment of litter (Ferguson *et al.*, 1998).

Reducing crude protein content in the feed with supplementation of limiting amino acids is one of the major strategies to reduce nitrogen emission and environmental protection in intensive poultry farming (Moran *et al.*, 1992). The supplementation of exogenous protease is another strategy for increasing digestibility of crude protein, thereby reducing nutrient excretion in faeces (Mahmood *et al.*, 2017), and deactivating anti-nutritional proteins, such as trypsin inhibitors (Ndazigaruye *et al.*, 2019). Treating the litter with methanotrophic bacteria like *Methylobacterium methanica* (MM) is yet another biological method of reducing nitrogen excretion from the litter. This gram-negative bacterium cleaves methane in a metabolic process called methanotrophy to obtain carbon and energy for its growth (Madigan and Martino, 2006); thus, reducing methane emission from poultry litter. These nitrogen excretion mitigation strategies have been very well studied in commercial broiler farming, but not adequately in intensive guinea fowl production. Therefore, the present study was carried out to determine the effect of low CP with supplemented amino acids and protease enzymes in the feed and MM litter

treatment on the production performance of guinea fowls in intensive farming.

MATERIALS AND METHODS

Three hundred and sixty day-old keets (*Numida meleagris*) were weighed, wing banded and allotted randomly into four treatments replicated thrice with 15 birds. The treatment groups were fed pre-brooder (0-4 weeks) and brooder diets (5 - 8 weeks) with high or low protein diets with or without fishmeal (FM) as given in Table 1.

T1 - High protein diet with FM

T2- High protein diet without FM

T3 - Low protein diet with FM+ protease enzyme

T4 - Low protein diet without FM+ protease enzyme

At the start of ninth week, three replicates in each treatment were additionally treated with a spray of 75 ml of MM bacterial culture on the litter at weekly intervals, adding four more treatments, making it eight treatment groups from 9 to 16 weeks of age.

T5 - High protein diet with FM + MM litter treatment

T6- High protein diet without FM + MM litter treatment

T7 - Low protein diet with FM+ Protease enzyme + MM litter treatment

T8 - Low protein diet without FM+ Protease enzyme + MM litter treatment

The seed culture of MM bacterium was procured from the American Type Culture Collection (ATCC), Bangalore, India. The seed culture was propagated in Nitrate Mineral Salts (NMS) liquid media having pH 6.8. The optical density (OD) of propagated bacteria was calculated using spectrophotometer and the culture having OD value of 0.082 was used for spraying on the litter.

The production performance of bi-weekly body weight was recorded on an individual basis. The feed consumption was recorded at bi-weekly intervals from all the replicates and the feed efficiency was calculated as the ratio of feed consumed to the body weight gained. The mortality was recorded on its occurrence and the livability was calculated on bi-weekly basis. The benefit-cost ratio of different feed treatments was calculated from the expenditure and income particulars.

All the data were subjected to statistical analysis as per Snedecor and Cochran, (1994) and the analysis was carried out by MS-Excel and IBM-SPSS (Version 20.0).

RESULTS AND DISCUSSION

Bi-weekly body weight

Perusal of bi-weekly body weight (Table 2) revealed that at the end of pre-brooder stage, (4th week), the keets fed with high protein diet containing FM (T1) had significantly ($P<0.05$) higher body weight than their counterparts fed high protein diet devoid of FM (T2) and low protein diets supplemented with protease enzyme or without FM (T3 and

T4 respectively). At the end of the brooder stage, that is, at the eighth week of age, bird received high protein diets (T1 and T2) attained significantly ($P<0.05$) higher body weight compared to that of low protein diets (T3 and T4), irrespective of addition of FM. At the end of feeding grower diet (16th week of age), high protein diet fed birds irrespective of FM addition or MM treatment (T1, T2, T5 and T6) attained significantly ($P<0.05$) higher body weight compared to the birds fed with low protein diets devoid of FM irrespective of MM litter treatment (T4 and T8).

The overall trend of biweekly body weight from the sixth week onwards revealed that the birds fed with high protein feed either containing FM (T1 and T5) or devoid of it (T2 and T6) performed similar ($P>0.05$), indicating that eliminating FM from the feed had no influence on growth rate of guinea fowls provided the CP and the amino acid levels are balanced. In a similar study, Mikulec *et al.* (2004) observed that replacing FM with soybean meal had no influence on either live weight or live weight gain of commercial broilers. Similarly, Aziz *et al.* (2001) also reported that the protein from FM can be replaced completely by soybean meal with the supplementation of 0.10% methionine. Chrystal *et al.* (2020a) found that the reductions in dietary CP from 20 to 17.2 % supported by inclusions of unbound essential amino acids did not compromise growth performance. The present study proved that low CP content coupled with the exclusion of FM can impair the growth rate with significantly ($P<0.05$) lower body weight

in guinea fowls throughout the study period from sixth weeks of age irrespective of MM treatment (T4 and T8).

The overall trend of body weight in the current study revealed that feeding guinea fowls with high protein diets (T1 and T5) had significantly ($P<0.05$) higher body weight compared to that of low protein diet fed birds (T3 and T7), irrespective of the addition of FM. This in contrast with most of the earlier reports; where, the 35th day body weight of commercial broilers fed with low protein feeds of 2% (Belloir *et al.*, 2017) and 3% (Benahmed *et al.*, 2023; De Rauglaudre *et al.*, 2023) less CP 56th day body weight of free range yellow broilers fed with 2% less CP (Shao *et al.*, 2018) were not affected when the requirements for amino acids (AA) were adequately met by supplementing synthetic AAs in reduced-CP diets.

In the present study, supplementation of protease enzyme in low protein diet did not restore ($P>0.05$) the body weight of guinea fowls compared to those receiving high protein diets; which is contradictory to the earlier reports of Rehman *et al.* (2018), Law *et al.* (2018) and Park *et al.* (2020) in commercial broilers who reported that the addition of exogenous protease enzyme in the LP diet from day-old to 35 days of age restored the body weight to that of control.

It was also noted in this study that MM treatment of litter had no influence on the body weight of guinea fowls compared to their counterparts receiving no MM treatment (T5 Vs. T1; T6 Vs. T2; T7 Vs. T3; T8 Vs. T4).

Feed consumption

The trend in bi-weekly feed consumption (Table 3) revealed no significant influence of CP level or addition of protease in feed or the MM litter treatment was noticed throughout the study period. The cumulative feed consumption up to 16 weeks of age of different treatments ranged within a narrow range from 5431 to 5498 g and the differences between treatments were not significant ($P>0.05$).

In the present study on guinea fowls, reducing the CP level in feed by 2% did not influence feed consumption. Although negative association (Ferguson *et al.*, 1998; Rehman *et al.*, 2018; Chrystal *et al.*, 2020a) between dietary protein level and feed consumption in commercial broilers has been reported in the literature, a different observation of no influence of feed consumption with decreasing level of dietary CP in commercial broilers has also been reported (Olomu and Offiong, 1980; Shao *et al.*, 2018; Belloir *et al.*, 2017; Benahmed *et al.*, 2023) similar to the findings of this study.

The current study revealed that the feed consumption of guinea fowls was not influenced by replacing FM with feed ingredients of plant origin similar to the findings of Aziz *et al.* (2001), who also reported similar findings when FM in commercial broiler ration was replaced with soybean meal.

The supplementation of protease enzyme also evinced no response in feed consumption of guinea fowls similar to the findings of Park *et al.* (2020) in commercial broilers. Whereas,

Rehman *et al.* (2018) found a significant ($P<0.05$) increase in the feed intake with protease supplementation.

Feed efficiency

The cumulative feed efficiency (Table 4) was not influenced by the CP level at the end of feeding the pre-brooder diet (4th week). As the experiment continued, at the end of the brooder period (8th week), the feed efficiency between high protein diet groups showed a significant ($P<0.05$) difference with the FM-containing group performing better than birds receiving a diet devoid of FM (T2). Similarly, between low protein diets, the FM group (T3) had better ($P<0.05$) feed efficiency compared to the non-FM group (T4). Over a long term of 16 weeks, Although the feed efficiencies of all the groups were found similar, the overall trend revealed that withdrawing FM had significant ($P<0.05$) influence on feed efficiency in high and low protein diets; however, supplementing protease enzyme in LP diets had no beneficial effect on feed efficiency in guinea fowls.

A vast majority of earlier researchers reported compromise in feed efficiency in commercial broilers due to reducing CP content by a milder level of 1.5 to 2% (Olomu and Offiong, 1980; Chrystal *et al.*, 2020a and 2020b). However, similar to the findings in this study in guinea fowls, no significant ($P<0.05$) effect in commercial broilers have also been reported with the reduction of CP by 2% (Shao *et al.*, 2018; Belloir *et al.*, 2017) and by 3% (Benahmed *et al.*, 2023), while balancing the AA levels by supplementing synthetic AAs in reduced-CP diets.

Table 1. Ingredient composition of treatment feeds

Ingredients Percent	Pre-brooder (0 – 4 weeks)				Brooder (5 – 8 weeks)				Grower (9 – 16) weeks			
	T1	T2	T3	T4	T1	T2	T3	T4	T1 & T5	T2 & T6	T3 & T7	T4 & T8
Ingredient composition (for 100 kg)												
Maize	35.50	35.50	42.00	42.00	52.00	52.00	58.00	58.00	53.50	53.50	60.00	60.00
Peral millet	15.00	15.00	15.00	15.00	10.00	10.00	10.00	10.00	15.00	15.00	15.00	15.00
Soyabean meal	35.10	43.30	30.30	37.40	26.00	32.20	21.30	26.40	22.00	26.00	16.70	20.20
Dry Fish	8.00	-	7.00	-	6.00	-	5.00	-	4.00	-	3.50	-
Mineral mixture (BIS, 2007)	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
DCP	1.60	2.40	1.80	2.50	1.60	2.20	1.80	2.30	1.60	2.00	1.70	2.10
Salt	-	0.50	-	0.50	2.10	2.03	1.35	1.35	-	0.40	-	0.40
Oil	2.52	2.50	1.66	1.66	-	0.40	-	0.40	1.60	1.70	0.70	0.70
Paddy husk	1.48	-	1.44	0.14	1.50	0.37	1.75	0.75	1.50	0.10	1.60	0.20
Calcite	-	-	-	-	-	-	-	-	-	0.5	-	0.60
Total	100	100	100	100	100	100	100	100	100	100	100	100
DL - Methionine	30g	80g	60g	110g	50g	80g	80g	110g	30g	60g	60g	80g
Feed Additives***	605g	605g	605g	605g	605g	605g	605g	605g	605g	605g	605g	605g
Protease enzyme	-	-	50g	50g	-	-	50g	50g	-	-	50g	50g
Nutrient composition												
CP (%)	24	24	22	22	20	20	18	18	18	18	16	16
ME (K cal)	2900	2900	2900	2900	3000	3000	3000	3000	3000	3000	3000	3000
Lysine (%)	0.97	0.97	0.97	0.97	0.93	0.93	0.93	0.93	0.82	0.82	0.82	0.82
Methionine (%)	0.45	0.45	0.45	0.45	0.40	0.40	0.40	0.40	0.35	0.35	0.35	0.35

Table 2. Effect of different dietary proteins, enzyme supplementation and *Methylomonas methanica* bacteria on bi-weekly body weight (g) of guinea fowl keets

Treatment	Age (weeks)							
	2 (n=90)	4 (n=90)	6 (n=90)	8 (n=90)	10 (n=45)	12 (n=45)	14 (n=45)	16 (n=45)
T1-HPFM	93.97 ± 0.95	222.06 ^b ± 3.19	422.95 ^b ± 4.31	635.74 ^b ± 6.48	837.48 ^{cd} ± 8.69	1038.65 ^c ± 8.96	1245.69 ^c ± 10.25	1410.56 ^c ± 12.50
T2-HP	94.81 ± 1.04	219.40 ^{ab} ± 2.86	419.97 ^b ± 4.16	630.28 ^b ± 5.41	828.27 ^{bcd} ± 7.66	1040.17 ^c ± 8.51	1237.96 ^c ± 9.93	1408.72 ^{bc} ± 11.43
T3-LPFM+E	92.98 ± 0.73	211.69 ^a ± 2.66	406.47 ^a ± 5.15	609.33 ^a ± 5.72	804.88 ^{ab} ± 9.80	1002.13 ^a ± 8.85	1197.73 ^{ab} ± 9.57	1373.47 ^{abc} ± 10.64
T4-LP+E	91.47 ± 0.79	212.92 ^a ± 2.27	402.50 ^a ± 3.99	604.71 ^a ± 5.19	797.66 ^a ± 8.42	1003.11 ^a ± 7.21	1183.24 ^a ± 11.86	1359.30 ^a ± 13.50
T5-HPFM+MM					836.13 ^{cd} ± 8.72	1038.98 ^c ± 11.28	1241.70 ^c ± 13.65	1412.44 ^c ± 13.85
T6-HP +MM					852.56 ^d ± 9.10	1030.87 ^{bc} ± 6.80	1221.28 ^{bc} ± 9.60	1397.98 ^{abc} ± 12.09
T7-LPFM+E+MM					813.62 ^{abc} ± 10.44	1011.35 ^{ab} ± 9.78	1196.94 ^{ab} ± 11.48	1370.51 ^{ab} ± 13.34
T8-LP+E+MM					810.49 ^{abc} ± 9.76	1008.02 ^{ab} ± 8.31	1180.40 ^a ± 10.95	1363.79 ^a ± 14.57
F Value	2.58 ^{NS}	3.26 [*]	5.10 ^{**}	7.12 ^{**}	4.30 ^{**}	3.74 ^{**}	5.94 ^{**}	3.09 ^{**}

NS- Not significant; * Significant (P<0.05); ** Significant (P<0.01)

Table 3. Effect of different dietary proteins, enzyme supplementation and *Methylobomonas methanica* bacteria on bi-weekly feed consumption (g) of guinea fowl keets

Treatment	Age (weeks)								Cumulative (0 – 16) (n=3)
	2 (n=6)	4 (n=6)	6 (n=6)	8 (n=6)	10 (n=3)	12 (n=3)	14 (n=3)	16 (n=3)	
T1-HPFM	116.15 ± 1.38	302.66 ± 11.18	565.54 ± 9.70	682.11 ± 13.96	747.86 ± 12.44	893.40 ± 22.65	1106.79 ± 14.13	1019.85 ± 27.97	5434.36 ± 86.04
T2-HP	119.27 ± 1.32	295.23 ± 8.21	575.86 ± 4.76	695.03 ± 6.72	753.44 ± 8.45	953.43 ± 33.00	1070.05 ± 61.53	1077.00 ± 46.70	5539.31 ± 138.67
T3-LPFM+E	115.00 ± 1.21	281.32 ± 6.91	562.37 ± 10.10	682.35 ± 8.93	738.98 ± 19.82	891.84 ± 31.38	1053.12 ± 46.74	1112.93 ± 48.14	5437.91 ± 135.80
T4-LP+E	111.86 ± 2.70	294.44 ± 6.87	552.64 ± 5.38	698.59 ± 6.29	758.12 ± 23.15	942.09 ± 21.40	978.11 ± 30.39	1125.19 ± 63.01	5461.04 ± 176.56
T5-HPFM+MM	-	-	-	-	742.20 ± 30.26	900.20 ± 36.14	1089.50 ± 57.52	1069.59 ± 15.21	5467.95 ± 44.89
T6-HP +MM	-	-	-	-	853.72 ± 20.50	809.83 ± 34.38	1029.41 ± 37.00	1119.91 ± 71.91	5498.26 ± 203.83
T7-LPFM+E+MM	-	-	-	-	783.56 ± 35.83	903.04 ± 63.14	1007.97 ± 30.46	1095.44 ± 111.59	5431.05 ± 319.42
T8-LP+E+MM	-	-	-	-	796.24 ± 39.90	919.65 ± 12.37	937.76 ± 37.25	1173.84 ± 21.58	5485.02 ± 58.22
F Value	0.11 ^{NS}	3.00 ^{NS}	1.10 ^{NS}	1.48 ^{NS}	0.46 ^{NS}	0.87 ^{NS}	0.22 ^{NS}	1.40 ^{NS}	1.62 ^{NS}

NS- Not significant; * Significant (P<0.05); ** Significant (P<0.01)

Table 4. Effect of different dietary proteins, enzyme supplementation and *Methylomonas methanica* bacteria on bi-weekly feed efficiency of guinea fowl keets

Treatment		Age (weeks)								Cumulative (0 – 16) (n=3)
		2 (n=6)	4 (n=6)	6 (n=6)	8 (n=6)	10 (n=3)	12 (n=3)	14 (n=3)	16 (n=3)	
T1-HPFM		1.88 ± 0.01	2.36 ± 0.02	2.81 ^a ± 0.01	3.20 ^a ± 0.01	3.70 ^a ± 0.05	4.44 ^a ± 0.03	5.35 ± 0.09	6.21 ± 0.07	3.83 ^a ± 0.08
T2-HP		1.90 ± 0.01	2.36 ± 0.02	2.87 ^b ± 0.02	3.30 ^b ± 0.01	3.80 ^{abcd} ± 0.06	4.50 ^{ab} ± 0.09	5.41 ± 0.09	6.31 ± 0.03	3.91 ^{abc} ± 0.03
T3-LPFM+E		1.91 ± 0.01	2.37 ± 0.01	2.88 ^b ± 0.00	3.36 ^b ± 0.01	3.79 ^{abc} ± 0.02	4.52 ^{abc} ± 0.03	5.38 ± 0.10	6.33 ± 0.01	3.94 ^{bc} ± 0.01
T4-LP+E		1.89 ± 0.01	2.42 ± 0.02	2.91 ^b ± 0.02	3.45 ^c ± 0.03	3.92 ^d ± 0.03	4.60 ^{bc} ± 0.02	5.46 ± 0.09	6.39 ± 0.06	4.00 ^c ± 0.07
T5-HPFM+MM						3.72 ^{ab} ± 0.04	4.45 ^a ± 0.03	5.36 ± 0.08	6.27 ± 0.04	3.85 ^{ab} ± 0.04
T6-HP +MM						3.85 ^{cd} ± 0.03	4.54 ^{abc} ± 0.05	5.41 ± 0.01	6.34 ± 0.02	3.91 ^{abc} ± 0.02
T7-LPFM+E+MM						3.82 ^{bcd} ± 0.01	4.57 ^{abc} ± 0.02	5.43 ± 0.07	6.31 ± 0.01	3.94 ^{bc} ± 0.01
T8-LP+E+MM						3.90 ^{cd} ± 0.03	4.66 ^c ± 0.03	5.44 ± 0.07	6.40 ± 0.06	4.00 ^c ± 0.07
F Value		0.88 ^{NS}	1.39 ^{NS}	5.21 ^{**}	21.16 ^{**}	4.45 ^{**}	3.04 [*]	0.24 ^{NS}	1.98 ^{NS}	3.11 [*]

NS- Not significant; * Significant (P<0.05); ** Significant (P<0.01)

Table 5. Effect of different dietary proteins, enzyme supplementation and *Methylobionas methanica* bacteria on benefit-cost ratio (BCR) of guinea fowl rearing

Treatment	Production cost per bird (Rs.)	Returns per bird (Rs.)	Profit per bird (Rs.)	Production cost per kg live weight (Rs.)	Net profit per kg live weight (Rs.)	BCR
T1-HPFM	149.18	181.14	31.96	105.77	24.23	1.21
T2-HP	147.86	181.06	33.20	104.96	25.04	1.22
T3-LPFM+E	139.50	180.72	41.22	101.56	28.44	1.30
T4-LP+E	137.64	178.24	40.60	101.07	28.45	1.29
T5-HPFM+MM	149.18	181.14	31.96	104.34	25.66	1.21
T6-HP +MM	147.44	181.06	33.62	105.46	24.54	1.23
T7-LPFM+E+MM	139.42	180.22	40.80	101.73	28.27	1.29
T8-LP+E+MM	138.65	178.30	39.65	101.67	28.33	1.29

Replacing FM with feed ingredients of plant protein, although had a significant ($P < 0.05$) negative impact on feed efficiency during the earlier part of the current study with guinea fowls, there was no significant difference was evident when the whole study period was considered; which is inconsistent to the earlier study of Aziz *et al.* (2001) who replaced FM with soybean meal in the ration and recorded no significant effect on feed efficiency in commercial broilers.

Barring the feed efficiency of a couple of bi-weekly periods in this study, in general, supplementing protease enzyme in LP diet did not affect feed efficiency of guinea fowls, which was in contrast to the overwhelming majority of earlier studies reporting significant improvement of feed efficiency in commercial broilers (Rehman *et al.*, 2018; Law *et al.*, 2018; Park *et al.*, 2020).

Livability

Barring T4, which recorded a livability of 95.60%, all other treatment groups registered a livability of 100%. As the livability is more than 95% in all treatment groups, it could be concluded that the level of CP or withdrawing FM or supplementing of protease enzyme in the ration had no detrimental effect on liveability; similar to the findings of Shao *et al.* (2018) in commercial broilers fed with varying dietary CP levels of 19, 18 and 17% and of Law *et al.* (2018) in commercial broilers supplemented with dietary protease enzyme. Interestingly, all the treatment groups received litter treatment with MM organisms recorded 100% livability, indicating the non-pathogenic nature of MM.

Benefit- cost ratio

The production cost per bird as well as per kg live weight basis in the present study was lower in LP diet-fed groups (T3, T4, T7 and T8) compared to that of HP groups (T1, T2, T5 and T6); mainly because of reduced inclusion of costlier protein feed resources (Table 5). On the other hand, replacing FM with plant protein sources in diets (T1 and T5 Vs. T6 and T6) had no impact on production cost. In general, LP rations with FM (T3 and T7) as well as without FM (T4 and T8) had higher benefit-cost ratio ranging between 1.29 and 1.30 compared to lower values from 1.21 to 1.23 in HP rations (T1, T2, T5 and T6). It can be concluded that when guinea fowls are reared for meat purposes in intensive system of management, 2% low CP, that is, 22% instead of 24% during pre-brooder, 18% instead of 20% during brooder and 16% instead of 18% is optimum for higher economic returns. de Rauglaudre *et al.* (2023) also suggested that CP content could be reduced by up to 3.0% without compromising growth performance and associated traits of economic importance in commercial broilers as long as the requirements for AA are met.

REFERENCES

- Araújo, I.C.S., Guamán, C.G., Sousa, L.S., Santos, H.J.B., Lopes, T.S.B., Costa, B.T.A. and Lara, L.J.C. (2023). Guinea fowl production in the world. *World's Poultry Science Journal*, **79**(2): 379 – 390.
- Aziz, M.A., Khandaker, Z.H. and Islam, M.M. (2001). Effect of replacing protein

- from fish meal with soybean on the performance of broiler chicken. *Indian Journal of Animal Nutrition*, **18**: 23 - 28.
- Belloir, P., Meda, B., Lambert, W., Corrent, E., Juin, H., Lessire, M. and Tesseraud, S. (2017). Reducing the CP content in broiler feeds: impact on animal performance, meat quality and nitrogen utilization. *Animal*, **11**: 1881 – 1889.
- Benahmed, S., Askri, A., de Rauglaudre, T., Létourneau-Montminy, M.P. and Alnahhas, N. (2023). Effect of reduced crude protein diets supplemented with free limiting amino acids on body weight, carcass yield, and breast meat quality in broiler chickens. *Poultry Science*, **102**(11): 103041.
- Bernacki, Z., Kokoszynski, D. and Bawej, M. (2013). Laying performance, egg quality and hatching results in two guinea fowl genotypes. *Archiv für Geflügelkunde*, **77**: 109 – 115.
- Chrystal, P.V., Moss A.F., Khoddami, A., Naranjo, V.D., Selle, P.H. and Liu, S.Y. (2020a). Effects of reduced crude protein levels, dietary electrolyte balance, and energy density on the performance of broiler chickens offered maize-based diets with evaluations of starch, protein, and amino acid metabolism. *Poultry Science*, **99**(3): 1421 - 1431.
- Chrystal, P.V., Moss, A.F., Khoddami, A., Naranjo, V.D., Selle, P.H. and Liu, S.Y. (2020b). Impacts of reduced-crude protein diets on key parameters in male broiler chickens offered maize-based diets. *Poultry Science*, **99**(1): 505 – 516.
- de Rauglaudre, T., Meda, B., Fontaine, S., Lambert, W., Fournel, S. and Letourneau-Montminy, M.P. (2023). Meta-analysis of the effect of low-protein diets on the growth performance, nitrogen excretion, and fat deposition in broilers. *Frontiers in Animal Science*, **4**. doi: <https://doi.org/10.3389/fanim.2023.1214076>.
- Ferguson, N.S., Gates, R.S., Taraba, J.L., Cantor, A.H., Pescatore, A.J., Ford, M.J. and Burnham D.J. (1998). The effect of dietary crude protein on growth, ammonia concentration, and litter composition in broilers. *Poultry Science*, **77**: 1481 – 1487.
- Issaka, Y.B. and Yeboah, R.W.N. (2016). Socio-economic attributes of guinea fowl production in two districts in Northern Ghana. *African Journal of Agricultural Research*, **11**(14): 1209 -1217.
- Law, F.L., Zulkifli, I., Soleimani, A.F., Liang, J.B. and Awad, E.A. (2018). The effects of low-protein diets and protease supplementation on broiler chickens in a hot and humid tropical environment. *Asian Australasian*

- Journal of Animal Sciences*, **31**: 1291 – 1300.
- Madigan, M.T. and Martino, J.M. (2006). Brock Biology of Microorganisms (11th ed.). Pearson Prentice Hall, NJ 07458, USA. p 136. ISBN 0-13-196893-9.
- Mahmood, T., Mirza, M.A., Nawaz, H. and Shahid, M. (2017). Effect of different exogenous proteases on growth performance, nutrient digestibility, and carcass response in broiler chickens fed poultry by-product meal-based diets. *Livestock Science*, **200**: 71 – 75.
- Mikulec, Ž., Mas, N., Mašek, T. and Strmotić, A. (2004). Soybean meal and sunflower meal as a substitute for fish meal in broiler diet. *Veterinarski Arhiv*, **74**: 271 - 279.
- Moran, Jr. E.T., Bushong, R.D. and Bilgili, S.F. (1992). Reducing dietary crude protein for broilers while satisfying amino acid requirements by least-cost formulation: live performance, litter composition and yield of fast-food carcass cuts at six weeks. *Poultry Science*, **71**: 1687 – 1694.
- Ndazigaruye, G., Kim, D.H., Kang, C.W., Kang, K.R., Joo, Y.J., Lee, S.R. and Lee, K.W. (2019). Effects of low-protein diets and exogenous protease on growth performance, carcass traits, intestinal morphology, cecal volatile fatty acids and serum parameters in broilers. *Animals*, **9**: 226.
- Olomu, J.M. and Offiong, S.A. (1980). The effects of different protein and energy levels and time of change from starter to finisher ration on the performance of broiler chickens in the tropics. *Poultry Science*, **59**(4): 828 - 535.
- Park, J.H., Lee, S.I. and Kim, I.H. (2020). The effect of protease on growth performance, nutrient digestibility, and expression of growth-related genes and amino acid transporters in broilers. *Journal of Animal Science and Technology*, **62**(5): 614 - 627.
- Rehman, Z.U., Kamran, J., El-Hack, M.E., Abd.Alagawany, M., Bhatti, S.A., Ahmad, G., Saleem, A., Ullah, Z., Yameen, R.M.K. and Ding, C. (2018). Influence of low-protein and low-amino acid diets with different sources of protease on performance, carcasses and nitrogen retention of broiler chickens. *Animal Production Science*, **58**: 1625 - 1631.
- Shao, D., Shen, Y., Zhao, X., Wang, Q., Hu, Y., Shi, S. and Tong, H. (2018). Low-protein diets with balanced amino acids reduce nitrogen excretion and foot pad dermatitis without affecting the growth performance and meat quality of free-range yellow broilers, *Italian Journal of Animal Science*, **17**(3): 698 – 705.
- Snedecor, D.W. and Cochran, W.G. (1994). Statistical methods. 9th Edition. Iowa State University Press, Ames, Iowa.