Determination of ileal endogenous flow and standardized ileal amino acid digestibility of rice DDGS in broilers

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Abstract

In the present study, ileal endogenous amino acid (EAA) losses were estimated in broiler chicks on 7th and 21st day with a nitrogen free diet (NFD) to determine standardized ileal digestibility of rice distiller dried grain with solubles (Rice DDGS). Day-old 240 broiler chicks were equally distributed into two phases i.e. 7th day phase and 21st day phase. In both the phases there were two treatment groups (T_1 and T_2) - each having four replicates of 15 birds. All the birds from 7th day phase were procured broiler pre-starter feed during first 2 days. Then they were assigned into dietary treatments, T_1 (NFD) and T_2 (Rice DDGS diet) till day 7. Similarly, all birds from 21st day phase were provided broiler starter diet up to 16 days after which assigned into similar dietary treatments like 7th day phase till day 21st. On 7th and 21st day respective all birds were killed to obtain ileal digesta. Ileal endogenous amino acid flow was determined in T_1, and apparent ileal amino acid digestibility (AIAAD) and standardized ileal amino acid digestibility (SIAAD) were calculated in T_2 group. The study concludes that endogenous ileal amino acid flow was found to be higher at 7th day compared to 21st day. Further, AIAAD and SIAAD of rice DDGS increased with growth of birds.

Key words: Broiler; Endogenous amino acid loss; Rice distiller dried grain with solubles; Standardized ileal digestibility.

Introduction

Broiler farming is one of the most rapidly expanding areas of the agricultural sector in India. During the past few decades, most broiler strains have shown a considerable increase in productivity, primarily stimulated by advances in genetics and nutrition. As far trend of body weight and feed efficiency is concerned, broilers can attain a body weight of around 2.5 kg in 35 days, with a feed conversion ratio of 1.5 (Barua et al., 2020). The major component of broiler rearing is poultry feed, which accounts for 70-75% of total recurring expenses. It is interesting to note that the feed cost is basically controlled by the cost of conventional protein ingredients. Among the conventional protein ingredients, soybean meal (SBM) is marked as one of the best and an important protein ingredient in broiler feed. In recent years, instability of its production, improper exports and increased demand have resulted in its scarcity, leading to its price skyrocket (Dinani et al., 2019). Hence, brewer's dried grain (Gobezie & Ambaw, 2024) and Distiller's dried grains with solubles (DDGS) (Sharyari et al., 2020) are included in poultry rations due to their ability to partially replace high protein ingredients like soybean meal, while reducing the need of the supplemental amino acids (Fries-Craft and Bobeck, 2019).

The DDGS represents the nutrients enriched byproducts obtained after dry milling of fermented cereal grains by selected enzymes and yeasts to produce ethanol. However, due to variations in growing conditions, ethanol processing methods and oil extraction, the nutrient composition of DDGS from various sources differs widely (Meloche et al., 2013; Rho et al., 2017). DDGS inclusion in the broiler diet has many advantages including its wide availability, adequate protein level, complementary amino acid profile and effectiveness in broiler chicken. Rice DDGS is more cost-effective option compared to the corn DDGS because it has a higher protein level and a lower price per unit. Incorporating rice DDGS into animal feed not only cuts down on feed expenses but also promotes better health and growth in animals, making it a favored option.

The influence of nutrient excretion on the environment, together with the necessity to lower feed costs, has prompted the diet formulation, based on ileal digestible amino acids (AA) instead of excreta levels. The diet formulation, based on digestible amino acids (AA) can be accomplished by utilizing either the apparent or standardized ileal amino acid digestibility values. It is important to note that the AIAAD estimates do not take into account the involvement of endogenous amino acids, which necessitates estimation.

The standardized ileal amino acid digestibility (SIAAD) based diet formulation closely meet bird's requirements minimizing nitrogen excretion and reduce the variability in performance (Adedokum et al., 2015). Moreover, SIAAD content hugely affects poultry feed intake and growth rate (Zeng et al., 2015). The SIAAD is more effective than other ways to measure digestibility because it takes into account the amino acids that the animal produces for its metabolic needs, which are not absorbed at the end of the small intestine. On the other hand, faecal nitrogen digestibility, ignore these endogenous amino acid losses and can result in an overestimation of digestibility. The availability of literature regarding SIAAD of rice DDGS in broiler was very scanty hence it needs to assess urgently. Therefore, an attempt was made to estimate ileal endogenous amino acid losses and evaluate apparent and standardized ileal amino acid digestibility of rice DDGS in broiler chicken on 7th and 21st day.

Material and methods

Ethics statement

The present study was carried out at the Poultry experimental shed of Department of Animal Nutrition and all procedures used were in accordance with the approval of the ethical committee of WBUAFS, Kolkata, India.

Rice DDGS Collection

The rice DDGS were procured from IFB Agro Industries Ltd., Noorpur, Diamond Horbour Road, Dist. 24, South Parganas, West Bengal, India. The authenticated DDGS were prepared from rice crop only. The cost of the procured rice DDGS was Rs. 30/- per kg.

Diets and Experimental Design

Day old 240 broiler chicks were randomly equally distributed in completely randomized design to two experimental phases i.e. 7th day and 21st day phase. In both phases birds were randomly divided in two treatment groups (T_1 and T_2), each having four replicates of 15 birds. All birds from 7th day phase were offered broiler pre-starter feed during the first 2 days, then they were assigned into two dietary treatments T_1 (NFD) and T_2 (rice DDGS diet) till day 7. Similarly, all birds from 21st day phase were fed broiler diet up to 16 days, and then assigned into similar dietary treatments like 7th day phase till day 21st. Both the diets were formulated with slight modifications in NFD (having negligible protein) while rice DDGS diet (approximately 20% CP) having rice DDGS as sole source of protein. As an indigestible marker, titanium dioxide was incorporated at a concentration of 5 g/kg in the diet.

Table 1 Experiment diet formulation (g/kg as-fed basis)

S.No.	Ingredient (g/kg)	NFD	Rice DDGS diet
1	Corn starch	408	0
2	Dextrose	411	126
3	Purified cellulose	50	0
4	Soybean oil	50	50
5	Vit. Min. Premix	5	5
6	DCP	19	19
7	$NaHCO_3$	20	-
8	KCl	12	-
9	MgO	2	-
10	Choline chloride	3	3
11	Limestone	13	13
12	NaCl	2	2
13	Titanium Dioxide	5	5
14	Rice DDGS	0	777
Total		1000	1000

Estimation of Ileal endogenous amino acid

After completion of treatment periods all birds were euthanized on day (7^{th} and 21^{st}) respectively and contents of the complete ileum was collected, pooled (replicate wise) and freeze-dried at -20^0 C (Ravindran et al., 2017) followed by analysis of amino acids and titanium dioxide. Amino acids profile was analyzed using HPLC. The samples underwent initial hydrolysis using 6N HCL (which included phenol) for 24 hours duration at a temperature of 110 ± 20 °C within a glass tube that was sealed under vacuum. For estimation of methionine and cysteine amino acids, performic acid oxidation was performed before the acid hydrolysis. The amino acids in the hydrolysate were subsequently determined by HPLC after post column derivatization.

The titanium dioxide was incorporated into the experimental diet as an inert marker, which was subsequently analyzed in both the diets and ileal digesta (Short et al., 1996). An amount of 0.1 g of freeze-dried digesta /diet sample was subjected to ashing at 550 °C for 2 hours, followed by dissolution in 10 ml of 7.4 M sulphuric acid and boiling for 15 minutes. Further, 20 ml of hydrogen peroxide (30%) was added, leading to the characteristic golden orange color, the intensity of which was contingent upon the concentration of titanium. Aliquots of the resulting solutions, along with similarly prepared standard solutions, were analyzed using a UV spectrophotometer, measuring the absorbance at 410 nm. Ileal endogenous amino acid flow in birds fed NFD was calculated as milligrams of amino acid flow per 1 kg of DM intake (DMI) basis using the following formula (Moughan et al., 1992).

$$\text{Ileal amino acid flow, mg/kg of DMI} = [\text{amino acid in ileal digesta, mg/kg} \times (\text{diet Titanium dioxide, mg/kg/ileal Titanium dioxide, mg/kg})]$$

Calculation of AIAAD and SIAAD

Calculation of Apparent Ileal amino acid digestibility (AIAAD) was determined in birds fed rice DDGS diet by the under mentioned formula. The amino acid concentration and inert marker (titanium dioxide) was estimated before the calculation of apparent Ileal amino acid digestibility (AIAAD).

$$\text{AIAAD, \%} = [1 - (\text{Titanium dioxide in Rice DDGS diet/ Titanium dioxide in ileal digesta}) \times (\text{amino acid in digesta/amino acid in diet})],$$

Endogenous amino acid losses were considered for the correction of calculated AIAAD values which were in fact referred as SIAAD.

$$\text{SIAAD, \%} = \text{Apparent digestibility, \%} + [(\text{IEAA flow, g/kg of DMI})/ (\text{amino acid content of the rice DDGS diet, g/kg of DM})] \times 100.$$

Statistical analysis: The replicate pens were the experimental units and all data obtained were pooled replicate wise. The results were expressed as mean and standard error of mean. The data were subjected in the Statistical Package for Social Sciences (SPSS 21.0, Chicago, IL, USA).

Results and discussion

Endogenous Amino acid Flow

The quantity of endogenous amino acid (mg/kg DMI) flow in terminal ileum of birds fed NFD at 7th and 21st day was found higher at 7th day compared to 21st day of age. The amino acids namely, methionine (394 and 118), tryptophan (284 and 132) and cystine (379 and 165) were with the lowest values respectively on 7th and 21st day. In the ileal endogenous protein, the amino acids like, glutamic acid (1828 and 853), aspartic acid (1883 and 672), arginine (1459 and 445), serine (1150 and 598), leucine (1388 and 549) and valine (1180 and 542) were the most abundant amino acids at 7th and 21st day, respectively (Table. 2).

These findings in terms of quantity (mg/kg DMI) were not in accordance with previously reported values (Block and Dekker, 2017). The discrepancies in values might be due to its assessment at different ages, methods of its estimation and diet composition. The endogenous amino acid output from the basal ileum in broiler chickens is influenced by age, as a greater flow of amino acids was noted in younger chickens (5 days old) compared to those at 15 or 21 days of age (Barua et al., 2021). However, endogenous amino acid flow was less effective as the birds mature (Ravindran and Hendriks, 2004). This suggests that the flow of IEAA, as established in birds of a specific age, may lead to less precise standardized digestibility values when correcting the apparent AA digestibility estimates for broilers of varying ages. Further, the efficiency of amino acid re-absorption and protein synthesis rate in the intestine of pigs was age dependent and affects the endogenous amino acid flow (Nyachoti et al., 2000). It is worth mentioning that in the present study, IEAA flows were found to be significantly superior than those estimated with 15 or 21-day-old broilers fed a protein-free diet by Adedokun et al. (2007). However, the lower values were noticed when compared with the values found in a research study for 39 days old birds which might be due to composition of different energy source in diet (Kong and Adeola, 2013).

In the present experiment, the AA profile of endogenous proteins was dominated by two amino acids namely, glutamic acid and aspartic acid, however, the lowest flow rates of amino acids were noticed in case of sulphur-containing AA. This is in tune with earlier findings established for growing chickens utilizing either a protein-free diet method or methods involving peptide elimination (Block and Dekker, 2017; Barua et al, 2021).

Apparent ileal digestibility of rice DDGS

The AIAAD (%) of rice DDGS in 7th day old chicks varied from 34.86 to 57.04 whereas the values ranged from 48.35 to 78.79 in 21st day age (Table. 2). Threonine, isoleucine and methionine digestibility were found lower and arginine and alanine were found higher at 7th day. Similarly, cystine, methionine and threonine digestibility were found lower and arginine and phenylalanine were found higher at 21st day. It seems that the apparent ileal amino acid digestibility of rice DDGS increased on the 21st day than the 7th day.

Amino acid digestibility can be affected by many factors including the age of birds and the type of feedstuff fed to the birds. It is reported that the digestibility coefficients of amino acids increased with age and varied with feedstuff (Bryden and Li, 2010). Age and the physiological state of the birds influence the ability to digest and absorb protein and amino acids because the small intestine has a lower enzymatic capacity to hydrolyze them at a young age. However, it has been reported that the apparent ileal amino acid digestibility of corn DDGS for both essential and non-essential amino acids ranged from 66% to 85% in broiler birds at 21day age (Adedokun et al, 2015). These values are slightly higher than our present finding (48.35% to 69.36%). However, the lysine digestibility was found to be slightly lower (58.5%) compared to our finding (67.15%).) The apparent ileal amino acid digestibility of light colour variety DDGS (at 5th and 21st day of age) had higher digestibility than dark colour variety DDGS (Adedokun et al., 2008). Maillard reaction might have been initiated on application of higher temperature for drying of DDGS which ultimately resulted in darker colour. This in turn decreased the concentration as well as digestibility of lysine. Because of the presence of free amino group, lysine is particularly susceptible to go through Maillard reactions, which simply reacts with reducing sugars. The amino acids digestibility of rice DDGS were found similar to dark colour DDGS (Adedokun et al., 2008). The findings of Bandegan et al. (2009) were also in tune with our results except for the lysine which was slightly higher than the present findings.

Standardized ileal digestibility of rice DDGS

The values of SIAAD as reported in this study were actually derived by correcting with EAA losses obtain on same age 7th and 21st day. The SIAAD (%) of rice DDGS on 7th day old chicks varied from 40.34 to 62.59 whereas it was from 48.56 to 80.50 in 21st day of age (Table. 2). Methionine, threonine and cystine digestibility were found to be lower and arginine and phenylalanine were recorded to be higher at 7th day. Similarly, cystine, methionine and

Table 2. EAA Loss, AIAAD and SIAAD of rice DDGS at 7th and 21st day of age in broiler birds

S.No.	Amino acid	Endogenous ileal amino acid loss (mg/kg of DM intake)			Apparent ileal amino acid digestibility of rice DDGS (%)			Standardized ileal amino acid digestibility of rice DDGS (%)		
		7th Day	21st Day	SEM	7th Day	21st Day	SEM	7th Day	21st Day	SEM
1	Alanine	799	396	13.29	51.01	67.57	0.78	54.31	69.46	0.69
2	Arginine	1459	445	11.40	57.04	78.79	1.18	62.59	80.50	1.18
3	Aspartic acid	1883	672	13.49	37.66	58.98	1.17	43.39	60.98	1.17
4	Cystine	379	165	12.95	37.18	48.35	0.68	41.50	48.56	0.68
5	Glutamic acid	1828	853	15.43	45.81	62.70	0.76	48.56	63.98	0.76
6	Glycine	941	378	16.56	39.29	67.72	1.17	44.76	65.92	1.17
7	Proline	759	480	14.82	45.53	63.55	0.94	48.98	65.76	0.92
8	Serine	1150	598	18.74	40.44	61.67	1.27	46.34	64.74	1.27
9	Tyrosine	587	358	19.27	49.72	63.74	1.37	55.16	67.06	1.37
10	Histidine	440	207	15.13	44.43	65.28	1.00	47.54	67.22	1.00
11	Isoleucine	766	382	23.10	35.09	64.01	1.55	47.32	66.37	1.14
12	Leucine	1388	549	28.68	48.67	65.98	0.95	52.49	67.46	0.95
13	Methionine	394	118	10.45	36.90	57.57	0.97	40.34	58.76	0.97
14	Phenylalanine	771	369	12.33	55.24	69.36	1.64	56.51	71.15	1.24
15	Threonine	800	628	15.02	34.86	57.63	1.08	40.45	62.02	1.08
16	Tryptophan	284	132	7.95	46.63	63.14	1.44	53.58	66.22	1.49
17	Valine	1180	542	11.65	42.52	64.77	0.76	47.72	67.16	0.76
18	Lysine	822	360	18.07	38.36	67.15	1.23	45.28	70.18	1.24

aspartic acid digestibility were found to be lower and arginine and phenylalanine were found to be higher at 21st day. Similar to the AIAAD, SIAAD of rice DDGS also increased at 21st day compared with 7th day. The improvement of SIAAD was approximately 19% and 15.5%, respectively on 7th and 21st day than the AIAAD. The higher improvement was observed in the case of lysine and glycine amino acid while cystine is the least once.

At 21st days of age, the SIAAD values for rice DDGS were slightly lower than the findings of Adedokun et al. (2015). Similarly, the SIAAD of light colour DDGS on the 21st day was reported to be higher (Adedokun et al., 2008) compared to our finding at the same age, however, the SIAAD of dark colour DDGS was quite similar to our finding. The differences in the digestibility of amino acids, particularly lysine, methionine, threonine, and cystine in maize DDGS, were chiefly attributed to the high temperatures used during the drying processes, which could have led to the darker coloration of the DDGS (Świątkiewicz and Koreleski, 2008). The estimated SIAAD of wheat DDGS in broilers at 21-day age had much more variable amino acid digestibility of than the variability of amino acid content (Cozannet et al., 2011). It is reported that SIAAD of lysine was found to be 46% lower (Cozannet et al., 2011) when compared with our finding and SIAAD of corn DDGS reported earlier.

Conclusion

The study concluded that the endogenous amino acid losses were higher at 7th day. Therefore, 21st day age will be more suitable for its estimation in broiler birds. In endogenous flow glutamic acid and aspartic acid were abundant while methionine, tryptophan and cystine were lower on quantity basis. Both AIAAD and SIAAD of rice DDGS increased at 21st day compared to the 7th day.

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