Apparent crude protein digestibility of soyabean meal estimated from ileal digesta of intact and excreta of caecectomised laying hens

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Abstract

Ileal digesta collection from intact birds is widely regarded as a reliable method for estimating CP digestibility; however, it is labour intensive and susceptible to hindgut microbial fermentation. Caecectomy attenuates caeca microbial fermentation, thereby enabling excreta to represent ileal nutrient disappearance more precisely. Therefore, this study evaluated the ileal digestibility of crude protein (CP) from soybean meal (SBM) in intact and caecectomised laying hens. The experimental diets comprised a control diet without SBM and four test diets in which SBM replaced cassava starch at 50, 100, 150, and 200 g/kg. Titanium dioxide (5 g/kg) was included as an indigestible marker. A total of 20, 18-week-old Bovans Nera hens with an average weight of 1.65 kg were individually caged, ad libitum access to layers mash and water until 26 weeks of age and were randomly allocated to the five diets. The birds were fed for seven days, after which they were weighed, euthanized by CO_2 asphyxiation, and ileal digesta from the distal two-thirds of the ileum were collected, frozen, and freeze-dried for analysis. The second group of 20, 16-week-old Bovans Nera hens, weighing 1.3 kg, was also housed individually with free access to layers mash and water. At 23 weeks, the birds were surgically caecectomised and allowed to heal for three weeks. At 26, the caecectomised birds were randomly assigned to the five test diets. After seven days on the diets, excreta was collected on a cage basis, pooled, frozen, and freeze-dried for analysis. Feed intake was significantly ($P < 0.05$) higher in caecectomised hens (1513.38 kg) when compared with intact hens (872.78 kg). However, SBM inclusion level had no significant effect on CP digestibility in either group. Mean CP digestibility values for SBM were comparable between intact (95%) and cecectomized hens (96%). In conclusion, caecectomised laying hens are a suitable model for CP digestibility studies, as excreta can be used for reliable estimates, and the birds can be employed.

Keywords: Caecectomised birds, Intact birds, Crude protein, Digestibility, Amino acid

Apparent crude protein digestibility of soybean meal in intact and caecectomised laying hens

Digestibilité apparente des protéines brutes du tourteau de soja estimée à partir du contenu iléal de poules pondeuses intactes et des excréments de poules pondeuses ayant subi une cæctomie

Résumé

Le prélèvement du contenu iléal chez des oiseaux intacts est largement considéré comme une méthode fiable pour estimer la digestibilité des protéines brutes ; cependant, cette méthode est laborieuse et sensible à la fermentation microbienne dans l'intestin postérieur. La cæctomie atténue la fermentation microbienne dans le cæcum, ce qui permet aux excréments de refléter plus précisément la disparition des nutriments iléaux. Par conséquent, cette étude a évalué la digestibilité iléale des protéines brutes (PB) provenant de la farine de soja (SBM) chez des poules pondeuses intactes et caécectomisées. Les régimes alimentaires expérimentaux comprenaient un régime témoin sans SBM et quatre régimes test dans lesquels la SBM remplaçait l'amidon de manioc à raison de 50, 100, 150 et 200 g/kg. Du dioxyde de titane (5 g/kg) a été ajouté comme marqueur indigestible. Au total, 20 poules Bovans Nera âgées de 18 semaines et pesant en moyenne 1,65 kg ont été placées individuellement en cage, avec accès à volonté à une pâtée pour poules pondeuses et à de l'eau jusqu'à l'âge de 26 semaines, puis réparties de manière aléatoire entre les cinq

régimes alimentaires. Les oiseaux ont été nourris pendant sept jours, après quoi ils ont été pesés, euthanasiés par asphyxie au CO_2 et le contenu digestif des deux tiers distaux de l'iléon a été prélevé, congelé et lyophilisé pour analyse. Le deuxième groupe, composé de 20 poules Bovans Nera âgées de 16 semaines et pesant 1,3 kg, a également été logé individuellement avec libre accès à une alimentation pour poules pondeuses et à de l'eau. À 23 semaines, les oiseaux ont subi une cécectomie chirurgicale et ont été laissés en convalescence pendant trois semaines. À 26 semaines, les oiseaux ayant subi une cécectomie ont été répartis de manière aléatoire entre les cinq régimes alimentaires testés. Après sept jours de régime, les excréments ont été collectés cage par cage, regroupés, congelés et lyophilisés pour analyse. La consommation alimentaire était significativement ($P < 0,05$) plus élevée chez les poules ayant subi une cécectomie (1 513,38 kg) que chez les poules intactes (872,78 kg). Cependant, le niveau d'inclusion de SBM n'a eu aucun effet significatif sur la digestibilité des protéines brutes dans les deux groupes. Les valeurs moyennes de digestibilité des protéines brutes pour le SBM étaient comparables entre les poules intactes (95 %) et les poules caécectomisées (96 %). En conclusion, les poules pondeuses caécectomisées constituent un modèle approprié pour les études sur la digestibilité des protéines brutes, car les excréments peuvent être utilisés pour des estimations fiables et les oiseaux peuvent être utilisés.

Mots-clés: Oiseaux caécectomisés, Oiseaux intacts, Protéines brutes, Digestibilité, Acides aminés

Introduction

Crude protein (CP) digestibility remains a central metric for evaluating protein quality and amino acid availability in poultry feed ingredients. Modern analytical approaches have expanded the capacity to estimate nitrogen and amino acid digestibility, with both ileal sampling and excreta collection commonly employed in research and industry settings. Recent studies, such as Ajao *et al.* (2024), have utilized ileal digesta collection to compare apparent ileal amino acid digestibility in broiler chickens fed low-protein diets supplemented with alternative protein sources. Similarly, O'Lear Reid *et al*. (2024) assessed the ileal digestibility of Spirulina in broilers and laying hens, demonstrating the continued relevance of direct digesta-based methodologies. While excreta-based assays remain widely used due to their simplicity, they are confounded by extensive microbial fermentation within the avian caeca, where bacterial populations degrade undigested protein and uric acid. Classic work by McNab, 1973; Parsons, 1986; Mead, 1989; established that microbial protein can constitute approximately 25% of total excreta protein, thereby altering amino acid profiles and biasing estimates of digestibility. This microbial interference is well documented to distort both CP and amino acid digestibility values derived from total excreta (Parsons *et al*., 1982; Ravindran and Bryden, 1999).

To improve accuracy, ileal digesta collection is often recommended, as it minimizes post-ileal microbial modification of amino acids (Ravindran *et al*., 1999). However, traditional ileal sampling approaches require euthanasia of large numbers of birds, while ileostomy and cannulation, though repeatedly tested, are associated with technical challenges, leakage, risk of infection, and high welfare costs. These limitations have driven interest in alternative models, particularly caecectomised birds, which eliminate the cecal microbial contribution to nitrogen recycling. Recent investigations highlight the value of this model. For example, Siegert *et al.* (2024) demonstrated that caecectomised laying hens yield consistent and reliable amino acid digestibility values across multiple soybean meal sources, especially for first-limiting amino acids. Likewise, the precision-fed caecectomised rooster assay has been validated, with Smola *et al.* (2025) confirming that standardized ileal digestibility (SID) values obtained from caecectomised birds are additive across mixed diets, an essential criterion for feed formulation. Additional work involving nutrient disappearance in caecectomised hens fed diets with varying mineral supplementation (e.g., monocalcium phosphate and phytase) further substantiates the model's reliability while reducing the confounding effects of cecal fermentation.

Collectively, the emerging body of evidence supports the use of caecectomised laying birds as an accurate, precise, and welfare-compatible alternative for determining CP and amino acid digestibility. This model effectively circumvents the microbial distortion inherent in excreta-based measurements and avoids the surgical complexity and ethical concerns associated with ileal cannulation. Thus, caecectomised birds can serve as a practical method of choice for high-precision assessment of protein digestibility in poultry nutrition research.

Materials and methods

Experimental site

The surgical procedure for caecum removal was done at the veterinary clinic in the Faculty of Veterinary Medicine, and the digestibility experiment was done at the Teaching and Research Farm, all situated within the University of Ibadan.

Birds and their management

Forty 18-week-old point-of-lay Bovan Nera layer birds (20 caecectomised and 20 intact) with a mean body weight of 1.5 ± 1.8 kg were housed in individual cage compartments at the Poultry Unit of the Teaching and Research Farm, University of Ibadan.

Experimental diets were commenced at 26 weeks of age. Hens were offered 120g/d of feed, and feed residues were collected daily. Weekly feed intake for each bird was recorded.

Intact birds

The intact birds were asphyxiated with carbon dioxide on the seventh day, and the section between Meckel's diverticulum and 2cm anterior to the ileo-ceca-colonic junction was removed. The digesta from two-thirds of this section was obtained for analysis as earlier suggested (Rezvani *et al.,* 2008). Digesta was gently flushed out with distilled water, frozen immediately, and later freeze-dried.

Caecectomised birds

Hens were caecectomised at 20 weeks through ventral abdominal laparotomy incision and excision of the ceca at the ileocecal junction under general anaesthesia as described (Rezvani *et al.*, 2007). Samples of excreta were collected for 7 days, frozen, and dried for analysis.

Dietary Treatments

Five diets were used in both experiments. In Diet 1 (control), soybean meal was not included, while diets 2, 3, 4, and 5 included soybean meal at 5%, 10%, 15%, and 20%, respectively. Soybean meal replaced cassava starch in a 1:1 ratio, so that the differences in amino acid concentration between diets resulted from soybean. Titanium dioxide was included as an indigestible dietary marker at the rate of 5g/kg diet as shown in Table 1.

Analysis and Calculations

Diets, freeze-dried digesta, and excreta samples were finely ground with a mortar and pestle before analysis. Diets were analyzed for dry matter, ash, crude fibre, crude protein, crude fat, and titanium dioxide (TiO_2), while digesta and excreta samples were analyzed for TiO_2 and crude protein. Proximate composition of feed was determined according to AOAC (2005). Titanium dioxide concentrations in diets and digesta were measured spectrophotometrically following the method of Brandt and Allam (1987). Apparent digestibility in diet was calculated in both experiments according to the following equation:

Digestibility of CP or AA in Diet = $100-[(TiO_{2Diet} \times CP \text{ or } AA_{Digesta \text{ or } Excreta})/(TiO_{2Digesta \text{ or } Excreta} \times CP \text{ or } AA_{Diet})] \times 100$.

Where TiO_{2Diet} and $TiO_{2\ Digesta \text{ or } Excreta}$ = concentrations of TiO_2 in the diet and digesta or excreta samples (g/kg); and CP_{Diet} and $CP_{Digesta \text{ or } Excreta}$ = concentrations of CP in the diet and digesta or excreta samples (g/kg), as earlier stated (Rezvani *et al*., 2008). Calculations were made for each hen in the experiments.

Digestibility of crude protein in Soybean meal.

Daily crude protein (CP) intake (g/d) was calculated as feed intake multiplied by the analyzed CP content of the diet. The quantity of digested CP (g/d) was obtained as CP intake multiplied by its corresponding digestibility for each diet. Apparent CP digestibility of the protein source was estimated from the slope of linear regressions of the form $y = a + bx$, where x represented CP intake and y the digested CP, as described by Rodehutscord *et al*., (2004). The regression slope (b), multiplied by 100, was expressed as digestibility (%) and calculated using GraphPad Prism version 4.02 (GraphPad Software Inc., San Diego, CA, USA). Crude protein values from the test diets were compared

using a t-test, with a significance level for comparison at $P < 0.05$.

Table 1. Gross Composition of Experimental diets (g/100gDM)

	Soyabean inclusion levels (%)			
Ingredients	0	5%	15%	20%
Maize	43.0	43.0	43.0	43.0
Cassava starch	20.0	15.0	5.0	0.0
Soyabean meal	0.0	5.0	15.0	20.0
Groundnut cake	24	24	24	24
Fish meal	2.0	2.0	2.0	2.0
Common salt	0.5	0.5	0.5	0.5
Dicalcium phosphate	3.0	3.0	3.0	3.0
Oyster shell	6.25	6.25	6.25	6.25
Methionine	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25
Titanium dioxide	0.5	0.5	0.5	0.5
Total	**100**	**100**	**100**	**100**
Crude protein (%)	16.97	19.07	23.27	25.37
Metabolizable energy (cal/kg)	2807.42	2751.22	2721.22	2706.22
Methionine (%)	0.42	0.51	0.57	0.6
Calcium (%)	3.5	3.46	3.48	3.49
Phosphorous (%)	0.75	0.93	1.18	1.32
Lysine (%)	0.82	0.9	0.99	1.02

Results and Discussion

The results in Table 2 indicates distinct responses to dietary soybean meal (SBM) inclusion between intact and caecectomised subjects, reflecting differences in digestive capacity linked to hindgut function. In the intact group, 15% SBM produced the highest total value (1086.10), significantly greater than 0–10% inclusion, suggesting that moderate SBM levels optimize nutrient digestion when both foregut and hindgut fermentation are functional. Conversely, in caecectomised animals whose hindgut microbial activity has been removed the total values increased steadily with SBM level, as 20% SBM yielded the highest response (1568.03). The narrow SEM and highly significant P-value ($P < 0.001$) indicate a consistent linear effect, implying that in the absence of cecal fermentation, higher SBM inclusion directly enhances nutrient availability in the small intestine. These patterns align with established evidence that intact animals rely partly on hindgut fermentation for amino acid salvage and fiber breakdown (Zhang and Adeola, 2017), while caecectomised models exhibit digestion dependent almost entirely on pre-cecal enzymatic processes (Adeola and King, 2006). Additionally, previous studies have shown that moderate SBM levels optimize protein digestibility and minimize antinutritional effects in intact monogastric whereas animals with reduced microbial fermentation often benefit from higher-quality, highly digestible protein sources such as SBM (Stein *et al*., 2016). Overall, the present findings reinforce that optimal SBM inclusion varies with physiological digestive capacity, emphasizing the

interplay between protein level, gut functionality, and nutrient utilization efficiency.

Table 2: Total Feed Intake for Intact and Cecectomized Birds within 7 days

Parameters	Soyabean Meal Inclusion (%)					
	0	5	15	20	SEM	P value
Intact Total	872.78^{bc}	897.65^{bc}	1086.10^{a}	997.38^{ab}	49.7	0.0156
Caecectomised Total	1513.38^{c}	1524.08^{c}	1549.88^{ab}	1568.03^{a}	6.13	0

a, b, c: Means within the same row with different superscripts are significantly different ($P < 0.05$)

The crude protein (CP) content of the digesta did not differ significantly among the SBM inclusion levels ($P = 0.45$), as shown in Table 3. The dietary SBM between 0–20% did not substantially alter the protein concentration remaining in the digestive tract. This suggests relatively stable pre-cecal protein digestion efficiency across treatments, consistent with earlier observations that moderate variations in dietary protein source do not always reflect proportionate changes in digesta CP when endogenous secretions and microbial activity contribute to nitrogen passage (Stein *et al*., 2007). However, titanium dioxide (TiO_2), used as an inert digestibility marker, showed a significant reduction at 5% SBM ($P < 0.001$), implying altered digesta flow or marker recovery at this level. A lower TiO_2 concentration may indicate higher digesta dilution or increased passage rate, which aligns with findings that dietary protein sources can influence gastrointestinal transit dynamics (Adeola and King, 2006). The return to higher TiO_2 values at 10–20% SBM suggests stabilization of digesta flow at higher inclusion levels, possibly due to improved feed consistency or changes in fiber–protein interactions. Collectively, the results indicate that while SBM inclusion does not markedly affect digesta protein concentration, it may influence digesta kinetics at specific inclusion points, reinforcing the importance of evaluating both nutrient composition and marker behavior when interpreting digestibility outcomes.

Table 3: Percentage crude protein in the digesta

Parameter	Soyabean Meal Inclusion (%)					
	0	5	15	20	SEM	P value
%protein	22.77^{a}	16.58^{a}	21.52^{a}	27.63^{a}	4.17	0.45
TiO_2	12.09^{a}	8.27^{b}	12.07^{a}	12.92^{a}	0.68	0

a, b, c: Means within the same row with different superscripts are significantly different ($P < 0.05$)

The caecectomised laying birds showed a numerical increase in crude protein (CP) excretion as dietary SBM inclusion increased, from 33.68% at 0% SBM to 58.28% at 20%; however, the difference was not significant ($P = 0.19$) as shown in Table 4. This suggests that higher dietary protein levels may have exceeded the birds' pre-cecal digestive capacity, leading to greater urinary and faecal nitrogen losses, which is consistent with reports that caecectomised birds lacking hindgut fermentation excrete more nitrogen when dietary protein is not precisely balanced (Ravindran *et al*., 1999). Elevated CP in excreta at higher SBM levels also aligns with findings that excess dietary protein increases endogenous nitrogen excretion due to incomplete amino acid absorption or metabolic deamination (Alabi and Adedokun, 2025). Meanwhile, TiO_2 recovery was uniform across treatments ($P = 0.55$), indicating consistent marker passage and

confirming that the observed differences in CP excretion were metabolic rather than due to digesta flow irregularities. Overall, the pattern highlights that caecectomised birds require carefully balanced protein levels to minimize nitrogen wastage and optimize nutrient utilization, reinforcing established principles in monogastric nitrogen metabolism.

Table 4: Percentage crude protein in the excreta

Parameter	Soya bean Meal Inclusion (%)					
	0	**5**	**15**	**20**	**SEM**	**P value**
%protein	33.68[b]	46.93[ab]	48.86[ab]	58.28[a]	6.69	0.19
TiO_2	6.74[a]	3.91[a]	4.50[a]	4.49[a]	1.27	0.55

a, b: Means within the same row with different superscripts are significantly different ($P < 0.05$)

The digestibility coefficients for intact birds in Table 5 were uniformly high (99.48–99.62%) across diets, with no significant differences ($P = 0.70$). The uniformity of digestibility in intact birds aligns with previous findings that moderate adjustments in dietary protein sources do not drastically alter overall nutrient utilization when the gastrointestinal tract is fully functional (Stein *et al*., 2016). In contrast, caecectomised birds exhibited greater variability (83.92–98.33%) but similarly showed no statistically significant effect of SBM inclusion ($P = 0.42$). The lower digestibility value observed at 5% SBM suggests that the effect of caecectomy on amino acid digestibility is highly variable, as reported in the literature. This reduction may reflect a temporary disturbance in digesta transit time or nutrient absorption resulting from the absence of hindgut fermentation, a phenomenon previously documented in caecectomised poultry models (Ravindran and Bryden, 1999; Adeola and King, 2006). The subsequent recovery of digestibility at 10–20% SBM implies that higher protein inputs may help to offset the reduced fermentative capacity of caecectomised birds (Zhang and Adeola, 2017). Overall, the results indicate that while intact birds maintain consistently high digestibility across SBM levels, caecectomised birds show greater sensitivity to dietary changes, reflecting their dependence on efficient pre-cecal digestion for optimal nutrient utilisation. This emphasizes the utility of the precision-fed caecectomised rooster assay in evaluating protein quality (Smola *et al*., 2025).

Table 5: Digested crude protein of intact and caecectomised birds

	Soya bean meal Inclusion (%)					
Parameter	0%	5%	15%	20%	SEM	P value
Intact birds	99.48	99.48	99.62	99.58	0.09	0.7
Cecectomized birds	98.33	83.92	97.19	97.34	5.86	0.42

The regression analysis in Table 6 shows a near-perfect linear relationship between dietary SBM inclusion and the measured response in intact birds, as indicated by an R^2 of 1.0 and a relatively low residual standard deviation (Sy.x = 35). The slope (0.95 ± 1.1) suggests that proportional increases closely match increases in SBM inclusion in the response variable, while the moderate intercept value indicates a stable baseline when SBM is absent. The precision of this model reflects the uniform digestive efficiency of intact birds, whose enzymatic and microbial contributions to nutrient breakdown produce highly predictable nutrient absorption patterns. Comparable studies have shown similarly strong linear relationships in intact poultry when digestible protein sources are incrementally increased, highlighting the buffering capacity of the intact gastrointestinal tract (Stein *et al*., 2016).

In contrast, caecectomised birds exhibited a weaker, though still strong, linear association ($R^2 = 0.91$), accompanied by a substantially larger error term (Sy.x = 270) and greater variability in both intercept (–93 ± 830) and slope (0.96 ± 0.18). This increased variability reflects the

physiological consequences of cecal removal, particularly the loss of hindgut microbial fermentation, which normally stabilizes nutrient utilization and supports the recovery of endogenous amino acids (Zhang and Adeola, 2017). The high variation suggests that caecectomised birds depend heavily on pre-cecal digestion, making them more sensitive to fluctuations in diet composition and digestive enzyme activity. These results align with earlier findings (Ishibashi and Yonemochi, 2003; Rodehutscord *et al.,* 2004), and further support caecectomy as a valid, accurate, and efficient model for protein digestibility studies in poultry.

Table 6: Regression table illustrating the linear relationship between feed intake and bird response

Parameters	Intercept	Slope	R^2	Sy.x
Intact birds	-110 ± 72	0.95 ± 1.1	1	35
Cecectomized birds	-93 ± 830	0.96 ± 0.18	0.91	270

a, b: Means within the same row with different superscripts are significantly different ($P < 0.05$).

Conclusion

Caecectomised laying hens provide a reliable estimate of crude protein digestibility comparable to ileal digesta collection, minimising microbial interference; caecectomy allowed clearer differentiation among protein sources. Owing to its simplicity, reproducibility, and reduced bird requirements, it remains a valuable tool for precision protein digestibility studies in poultry nutrition.

Author contributions

Oluwatobi ONARINDE: Data curation; Writing-original draft preparation; writing-review, and editing; project administration**.**
David EYAREFE: Methodology, Surgery; investigation.
Eustace IYAYI: Conceptualiztion, methodology, writing-review, editing, project administration.
Akinyele ADESEHINWA: Writing, Reviewing, and Editing.

Data availability statement

The data presented in this study are available on request from the corresponding author.

Conflict of interest

The authors declare no conflict of interest

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