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Short communication

Effect of ileal sub-section and the method of collection of digesta on the determination of apparent ileal digestibility of amino acids in broilers

R. Poureslami [1], A.B. Batal [2], B. Jung *

Department of Poultry Science, The University of Georgia, Athens, GA 30602-2772, USA

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ABSTRACT

This study investigated the effect of the site of digestive sampling (terminal ileum *vs.* entire ileum) and the method of digesta collection (flushing *vs.* squeezing) on the determination of ileal amino acid (AA) digestibility in broilers. An experimental diet was formulated based on soybean meal and dextrose. The diet was fed to broiler chickens from 14 to 18 days of age. Twenty four replicate pens (twelve birds each) were given access to the experimental diet. Ileal contents were collected after euthanizing the birds by cervical dislocation at 18 days of age. Digesta were collected from the terminal (last 15 cm of the distal ileum) or entire ileum using either by flushing or squeezing with saline solution. The section from which the digesta were collected did not have a significant impact on the AA digestibility coefficients. However, values were higher ($P<0.01$) when based on digesta samples that were collected *via* flushing than digesta collected *via* squeezing. The results suggest that flushing procedure to determine AA digestibility is a more accurate procedure than squeezing the digesta from ileum. However, the digestive section (terminal or entire ileum) of collection of digesta did not affect the CAID of AA in broilers.

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1. Introduction

Protein is one of the most expensive components of poultry feed. Crude protein requirement is in fact the amino acid (AA) requirement thus the digestibility of AA in feedstuffs is an important feature of feed quality. The ileal AA digestibility assay has frequently been used to determine AA digestibility in broilers. In this assay, feed is offered to broilers for few days and at the end of the adaptation period, ileal contents are collected and analyzed for AA determination. Several sampling procedures have been used to collect the ileal digesta. In an early study, cannulation technique was used to collect digesta from the terminal ileum (Raharjo and Farrell, 1984). In a number of studies, digesta were flushed out from terminal or entire ileum using saline or distilled water (Kluth et al., 2005). In some other studies, digesta from the entire ileum were squeezed out (Bandegan et al., 2009). For feed evaluation purposes, it is of crucial importance that the results from different studies are comparable and can be standardized. To the best of our knowledge, no study has investigated the impact of the digesta sampling procedure on the AA digestibility determination. The objective of the present study was to investigate the effect

Abbreviations: AA, amino acid; AIA, acid-insoluble ash; CAID, coefficient of apparent ileal digestibility; CP, crude protein; N, nitrogen; TSAA, total sulfur amino acid; ME, metabolizable energy; SBM, soybean meal.

* Corresponding author. Present address: Department of Animal Science, Cornell University, Ithaca, NY 14853, USA. Tel.: +1 706 614 6555; fax: +1 706 542 1827.
E-mail address: letsgoala@gmail.com (B. Jung).
[1] Present address: Premier Nutrition Solutions LLC, Johnston, IA 50131, USA.
[2] Present address: Huvepharma, Inc., Peachtree, GA 30215, USA.

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Table 1
Composition of the experimental diet (g/kg as-fed basis).

Ingredient	
Soybean meal (480 g CP/kg)	458.8
Dextrose	467.0
Poultry fat	20.0
Dicalcium phosphate	18.5
Limestone	12.4
Sodium chloride	5.0
Vitamin premix[a]	2.5
Mineral premix[b]	0.8
Celite[c]	15.0
Total	1000.0
Calculated chemical analysis	
Metabolizable energy, MJ/kg	12.2
Analyzed chemical analysis	
Crude protein	216.0
Lysine	14.6
Methionine	3.2
Cysteine	3.3
Threonine	8.4
Arginine	16.6
Tryptophan	3.0
Valine	11.6

[a] Vitamin premix provided the following (per kilogram of diet): thiamin·mononitrate, 2.4 mg; nicotinic acid, 44 mg; D-Ca pantothenate, 12 mg; vitamin B_{12}, 0.01 mg; pyridoxine·HCl, 4.7 mg; D-biotin, 0.11 mg; folic acid, 5.5 mg; menadione sodium bisulfate complex, 3.34 mg; choline chloride, 220 mg; cholecalciferol, 27.5 μg; trans-retinyl acetate, 1892 μg; all-rac α tocopheryl acetate, 11 mg; ethoxyquin, 125 mg.
[b] Trace mineral premix provided the following (per kilogram of diet): manganese ($MnSO_4 \cdot H_2O$), 60 mg; iron ($FeSO_4 \cdot 7H_2O$), 30 mg; zinc (ZnO), 50 mg; copper ($CuSO_4 \cdot 5H_2O$), 5 mg; idodine (ethylene diamine dihydroiodide), 0.15 mg; selenium (Na_2SeO_3), 0.3 mg.
[c] Diatomaceous earth; Celite Corporation, Lompoc, CA.

of digestive section (entire *vs.* terminal ileum) and method of collection (squeezing *vs.* flushing) on the determination of AA digestibility in broilers.

2. Materials and methods

2.1. General procedures and diets

All experimental procedures were conducted in accordance with the University of Georgia Animal Care Committee. At hatch, Cobb 500 male broiler chickens were obtained from a local hatchery and placed in battery brooders (Petersime Incubator Co., Gettysburg, OH, USA) with raised wire floors in an environmentally controlled room. The initial temperature was 32 °C and reduced weekly by 2 °C. Chicks were fed a standard maize-soybean meal starter diet, adequate in all nutrients as recommended by National Research Council (1994), until 13 days of age. At 14 days of age, after fasting overnight, the chicks were weighed, divided into 24 replicate pens (twelve chicks each) and given access to the experimental diet. All birds had *ad libitum* access to feed and water and were maintained on 24 h lighting schedule. The experimental pelleted diet was formulated to contain soybean meal and dextrose (Table 1). Celite (Celite Corp., Lompoc, CA), was added as inert marker (acid-insoluble ash; AIA) at 15 g/kg of diet.

2.2. Sampling and analysis

At 18 days of age, birds were killed by cervical dislocation and digesta samples were collected from either the entire ileum or the terminal ileum. The entire ileum was defined as the segment of the intestine from Meckel's diverticulum to the ileo-cecal junction. A sub-section of 15 cm length of distal ileum was considered as the terminal ileum. Two methods were used to collect digesta, squeezing or flushing with saline (9 g/kg sodium chloride solution). Six replicate pens of broilers were assigned to one of the four aforementioned collection procedures. Ileum contents from ten birds out of twelve birds per pen were pooled into a plastic container and frozen at −20 °C, freeze-dried and finely ground with 0.5 mm sieve size using a coffee grinder for further analysis. Thus, ileal digesta in six samples per treatment and a total of 24 samples were analyzed for AA and AIA content. Feed and ileal digesta samples were submitted to a laboratory (Agriculture Experiment Station Chemical Laboratories, University of Missouri-Columbia, Columbia, MO, USA) to analyze total AA concentration. Crude protein ($N \times 6.25$) in the experimental diet was determined by procedure 990.03 of AOAC (2006). Amino acid content in the experimental diet and ileal digesta were determined by procedure 982.30 E (a, b, c) of AOAC (2006) using cation-exchange chromatography coupled with post-column ninhydrin derivatization. A subset of diet and ileal digesta samples was analyzed in duplicate for AIA as reported by Scott and Boldaji (1997). The coefficient of apparent ileal digestibility (CAID) was calculated as previously described (Garcia et al., 2007).