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Using acid-detergent insoluble ash as an internal marker to compare digestibility and fecal output by ovine that were offered hays of varying nutrient composition

Kennedy Barnett
University of Arkansas, Fayetteville, kdbarnet@uark.edu

Trisha Adams
University of Arkansas, Fayetteville, tma009@uark.edu

Kenneth P. Coffey
University of Arkansas, Fayetteville, kcoffey@uark.edu

Kathleen S. Jogan
University of Arkansas, Fayetteville, kjogan@uark.edu

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Cover Page Footnote

Kennedy Barnett is a May 2022 graduate with a degree in Animal Science. Trisha Adams is a May 2022 graduate with a degree in Animal Science. Dr. Kenneth P. Coffey, the faculty mentor, is a professor in the Department of Animal Science. Dr. Kathleen Jogan is an Instructor in the Department of Animal Science.

Using Acid-Detergent Insoluble Ash as an Internal Marker to Compare Digestibility and Fecal Output by Ovine that Were Offered Hays of Varying Nutrient Composition

Meet the Student-Authors

Kennedy Barnett

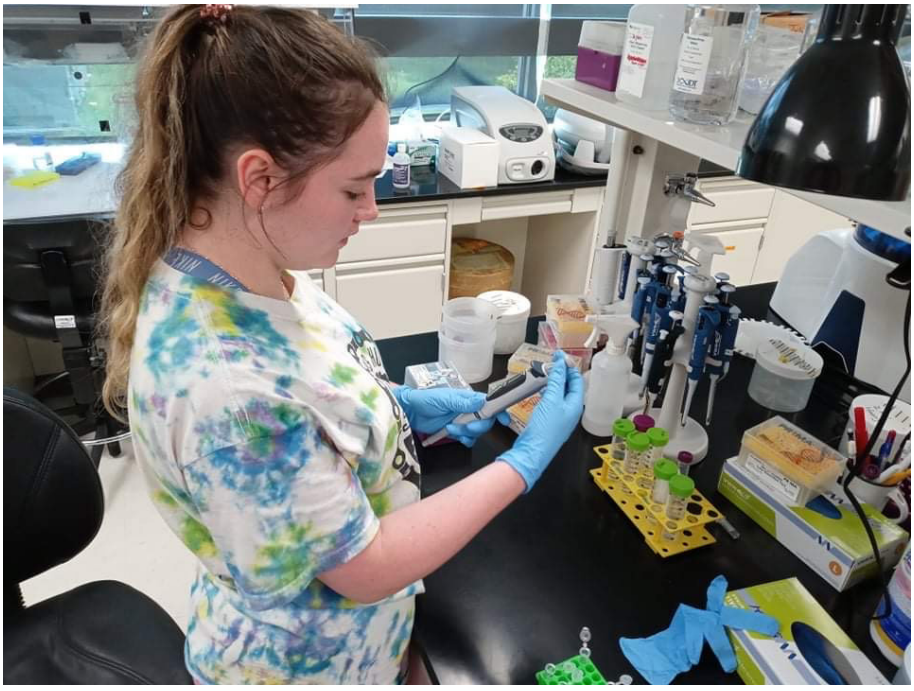
I graduated in May 2022 with a major in pre-professional animal science. I attended Northside High school in Fort Smith, Arkansas, where I was raised most of my life. In high school, I worked at a vet clinic through an internship course my high school offered. I was a member of the University's Pre-Vet Club, Women's club soccer team, and a volunteer at the University of Arkansas System Division of Agriculture's research farm in my spare time. I also volunteered for Elite Hospice. I currently am a veterinary assistant at Wedington Animal Hospital in Fayetteville, Arkansas, and hope to continue my education to earn a doctorate. I have only ever worked at vet clinics because it has always been my dream to become a large animal veterinarian. I have spent the last 4 years working to achieve my dream. My dream would be impossible if it were not for Dr. Kenneth Coffey and Dr. Kathleen Jogan, so I would like to thank them for being not only my professors but my mentors and for their unending support and feedback. Dr. Coffey never stopped helping me through every challenge, and it is because of him that I now have the skills needed to survive life and vet school.

Trisha Adams

I graduated in May 2022 with a major in Animal Science. I attended Greene County Tech High school in Paragould, Arkansas. I currently work as a veterinary assistant at Wedington Animal Hospital in Fayetteville, Arkansas. I am trained in surgical preparation, anesthesia, and recovery. I have assisted in an estimated thirty successful surgeries. I am well-versed in caring for hospitalized animals and performing medical treatments to help them recover from their illnesses. During my time as a veterinary assistant, I have re-homed seven pets that would have been left at a shelter or possibility euthanized due to behavior or health status. Two of those pets now live with me; one has overcome behavioral issues, and the other has been completely rehabilitated after a successful leg amputation. I also volunteered at the University of Arkansas school farm during the Dorper lambing season of 2021, which resulted in the lambing of over fifty Dorper lambs. Out of the fifty-plus lambs, we only had three bottle-fed lambs and a few lamb mortalities. This resulted in one of their most successful lambing seasons. My passion for animals inspired me to study research methods to improve the husbandry of farm animals. I would like to thank Dr. Kenneth Coffey and Dr. Kathleen Jogan for their wisdom and for this experience in laboratory research that helped me meet my ambitious goals.

Research at a Glance

- Our goal was to determine if an internal marker acid detergent insoluble ash could be used to replace total fecal collections.
- Acid detergent insoluble ash was an effective replacement for total fecal collections.
- Since total fecal collections are tedious and the marker can replace them, it saves researchers time and labor.



Kennedy in the nutrition lab learning the names and uses of the common instruments used to perform research. Using the tools before beginning research raised her confidence so when the real work began, she was no longer nervous she would mess up. She learned quickly that some tools seemed easy but in reality when used wrong could mess up all your data, especially when working with such small quantities.

Trisha practicing how to transfer liquids from one dish to another and getting used to safety protocols in the nutrition lab, along with how to use some of the basic research tools. Getting in practice before working with the real samples helped her to be consistent, safe, and to not contaminate anything. She was thankful to learn how the tools worked because in the future she could use those same tools for other research.

Using Acid-Detergent Insoluble Ash as an Internal Marker to Compare Digestibility and Fecal Output by Ovine that Were Offered Hays of Varying Nutrient Composition

Kennedy D. Barnett, Trisha M. Adams,† Kenneth P. Coffey,§ and Kathleen S. Jogan,‡*

Abstract

Digestibility markers are used to determine total digestibility without collecting total feces, which reduces time and labor and allows greater numbers of animal observations to be obtained. The goal of this research was to determine if the digestibility marker acid detergent insoluble ash (ADIA) was fully recovered and, based on that information, determine the accuracy of total digestion predictions using ADIA. Different proportions of sericea lespedeza hay and alfalfa silage were offered to 16 gestating Dorper ewe lambs (*Ovis aries*) (41.8 ± 4.61 kg body weight) to determine digestibility. Total feed intake and fecal excretion were measured during 2 experimental periods consisting of a 14-day adaptation period followed by 5 days of total fecal collection. The accuracy of ADIA recovery and its use to predict digestibility were analyzed using PROC MIXED of SAS. Recovery of ADIA averaged -0.16 g/d across treatments and was not different ($P = 0.97$) among treatments. Digestibility was underestimated by 6.2 g/kg, or 0.62 percentage units, and treatment differences were not detected ($P = 0.92$). Neither of these measurements was different ($P > 0.51$) from zero. Therefore, ADIA was proven to be a reliable internal marker to replace total fecal output for sheep offered forage-based diets with mixtures of diverse forages. Due to the recovery rate and total digestibility calculated, ADIA is a realistic and reliable procedure to determine total digestibility when total fecal collections are not feasible.

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Introduction

Indigestible internal markers are used across multiple species and diets. Plant wax N-alkanes have shown variable success as internal markers because although the marker is accurate for determining diet intake, it falls short of other ideal marker criteria (Dove and Mayes, 2005). Due to their long-chain alcohols and fatty acids, they are often not chemically different enough from other diet components, causing mistakes when trying to determine fecal output or total digestibility (Dove and Mayes, 2005). In species other than sheep, internal markers such as acid detergent lignin (ADL), acid-insoluble ash (AIA), and acid detergent insoluble ash (ADIA) are used across a variety of diets. No significant differences were observed in digestibility measurements between AIA and total collection in horses, indicating reliable success as an internal marker (Bergero et al., 2004) across diets of different hays and concentrates. However, digestibility estimated using ADL did not produce acceptable results (Bergero et al., 2004). Christian (2014) reported a high recovery of ADIA from beef cattle consuming forage diets with no supplements, making ADIA an effective marker in those situations. However, studies reporting ADIA recovery from sheep are limited. Furthermore, studies are limited that evaluate the recovery of ADIA when forage mixtures are offered or when forages containing anti-quality components that negatively impact digestion are offered. Therefore, this study was conducted to evaluate the recovery of ADIA from sheep offered diets composed of different combinations of alfalfa and sericea lespedeza to determine its usefulness as an internal marker to estimate digestibility in sheep.

Materials and Methods

Alfalfa used in the original study was grown at the University of Arkansas Milo J. Shult Agricultural Research and Extension Center (SAREC), located in Fayetteville, Ark. The alfalfa was chopped using a plot harvester machine into particles less than 5 cm and then allowed to wilt until moisture content was 60%. The alfalfa was then packed into plastic trash cans (132 L) lined with two plastic bags and stored for four months (Niyigena, 2019). The sericea lespedeza hay was obtained from a producer farm near Prairie Grove, Ark., and stored under a roof until feeding.

The twenty Dorper ewe lambs (41.8 ± 4.61 kg initial body weight) used in the original study were housed at the SAREC in individual 1 × 1.5-m pens with wire grate flooring in a climate-controlled barn. Other specific management practices, procedures for allocation to treatments, and specific feeding practices were reported by Niyigena (2019). Ewe lambs had ad libitum access to one of four diets: alfalfa silage alone or alfalfa silage with 9%, 18%, or 27% sericea lespedeza on a dry matter (DM) basis. All ewe lambs were also given 30 g/d of a commercial sheep mineral.

Each ewe lamb was given constant access to clean water and offered their respective diets for ad libitum consumption. The total feed amount was weighed daily, and the ewe lambs were given small portions throughout the day to minimize forage loss by spillage and to decrease the risk of forage selectivity. Orts were collected daily at 1600 and dried at 50 °C until reaching a constant weight. Fresh feed was offered immediately thereafter. Feed samples were collected daily for five days, beginning two days before total fecal collections, and dried at 50° C until reaching a constant weight. Pens were fitted with solid PVC trays positioned under each ewe lamb pen to collect all feces produced. Total feces were collected daily for five days beginning on day 15 of each period, weighed, and dried at 50 °C to reach a constant weight (Niyigena, 2019). As (Niyigena, 2019) states, the ewe lambs were all randomly assigned to 1 of 4 treatments. That provided 4 treatments in two periods. Each period was 14 days, followed by 5 days of total urine and feces collection with a 5-day break between periods. During the second period, ewe lambs were randomized to treatments with the condition that they were not offered the same treatment they were offered during the previous period. Ewe lambs were then removed for 5 days between periods and offered only alfalfa silage.

Samples were obtained from a total of 32 total collection observations. However, two samples were contaminated and were therefore discarded, resulting in 30 digestibility measurements to use for marker recovery determination. Approximately 0.50 g of each feed, fecal, and ort sample was analyzed in duplicate to determine marker recovery. Samples were placed into labeled Ankom bags and sealed using a heat press. Next, up to 25 Ankom bags were placed evenly into a container and placed into an Ankom 200 Fiber analyzer (ANKOM Technology Corporation, Macedon, N.Y., USA). The machine was filled with 120 mL of acid detergent fiber (ADF) solution, and the samples were submerged in the boiling ADF solution for 60 minutes. The ADF solution was then drained, and the bags were washed five times with boiling deionized water at five-minute intervals with rinse water removed between each rinsing. The samples were removed from the container and placed into a breaker to press out any excess liquid. Afterward, 50 mL of pure acetone was placed into the beaker with the samples to sit for 10 minutes to enhance evaporation of excess moisture. Next, the acetone was drained from the beaker, and the samples were spread evenly on a metal tray that was placed under a vent hood. The samples were allowed to dry for 10 minutes to ensure residual acetone was removed. Once samples were

dry to the touch, they were placed into a gravity-convection oven at 100 °C overnight to remove any remaining moisture. The next day, samples were removed and placed into a desiccator that was vacuum-sealed. Samples were weighed after reaching ambient temperature, placed in individual metal ashing trays, then placed into a muffle furnace at 600 °C for 8 h. Finally, the samples were allowed to cool to ambient temperature in a vacuum-sealed desiccator (Thiex et al., 2012).

The actual amounts of each feed component offered, the feed refused, and the total fecal output were multiplied by their respective concentrations of ADIA (g/kg DM) or (% DM). The ADIA intake was calculated as the amount (g/d) of ADIA offered minus the ADIA (g/d) in the orts. The fecal ADIA output was then subtracted from ADIA intake to determine ADIA recovery. Digestibility was estimated using the equation:

$$100 - \left[100 \times \left(\frac{\text{ADIA conc. in the feed}}{\text{ADIA conc. in the feces}}\right)\right]$$

where the concentration of ADIA in the feed was calculated as the total ADIA intake (g/d) divided by the actual dry matter (DM) intake. This calculation was necessary because the animals were offered their respective diets for ad libitum consumption, and substantial diet component selectivity occurred.

Actual digestibility, the difference between ADIA offered and ADIA recovered in the feces, and the difference between actual DM digestibility as estimated by collecting total feces and that estimated using ADIA were analyzed using PROC MIXED of SAS v. 9.4 (SAS Institute, Inc., Cary, N.C., USA). Data were analyzed as a randomized complete block design with the individual animal as the experimental unit. Treatments were considered fixed effects, while ewe and period were treated as random effects. Pairwise F-protected *t*-tests were used to determine differences among treatments. The probability greater than *t* was used to determine if the individual treatment means were different from zero ($P < 0.05$). All means are reported as least-squares means.

Results and Discussion

The neutral detergent fiber inclusive of ash (aNDF), ADF, condensed tannin, and ADIA concentrations were greater from sericea lespedeza than alfalfa, but N concentrations were greater from alfalfa (Table 1). The aNDF concentrations were greater (64.4% vs. 34.1% DM), and condensed tannin concentrations were slightly lower (16.8% vs. 18.2% DM) from the sericea lespedeza hay used in the present study compared with sericea lespedeza pellets in a previous study (Kronberg et al., 2018).

Dry matter digestibility was greatest ($P < 0.05$) with ewe lambs offered the diet of only alfalfa silage (Table 2). Digestibility was lower ($P < 0.05$) with ewe lambs offered the diet of 27% sericea lespedeza compared with

Table 1. Chemical composition of alfalfa silage and sericea lespedeza (SL) fed to ewes.[†]

Item	Alfalfa	Sericea lespedeza
Dry Matter, % fresh weight	42.3	93.6
	% of Dry Matter	
Organic Matter	91.0	87.2
Neutral Detergent Fiber Inclusive of Ash	38.1	64.4
Acid Detergent Insoluble Ash	31.9	56.1
Nitrogen	3.9	2.4
Condensed tannins	0.00	16.8
Acid Detergent Insoluble Ash	0.72	1.1

[†] Alfalfa silage was either offered alone (0 % CONT) or mixed with chopped SL to provide 9 (LOW), 18 (MED), or 27% SL (HIGH) on a dry matter (DM) basis.

those offered 18% sericea lespedeza, but overall, digestibility was not different ($P = 0.57$) between those offered 9% and 27% sericea lespedeza in the diet. Kronberg et al. (2018) reported stepwise decreases in DM digestibility of alfalfa hay-based diets as alfalfa was replaced with sericea lespedeza pellets.

Using diets with varying nutrient composition is a frequent practice for digestion studies regardless of animal species or feed (Bergero et al., 2004; Christian, 2014). This is done to determine if interactions among feedstuffs are linear or if possible associative effects are present. In the current study, both fiber and tannin concentrations increased with each increase in the percentage of sericea lespedeza in the diet. However, digestibility did not decrease linearly, thereby indicating possible associative effects.

The difference between ADIA offered and that recovered in the feces averaged -0.15 (g/d) across treatments, and none of the treatment means were different from zero ($P \geq 0.63$). There were also no differences ($P = 0.97$) among treatments in ADIA recovery. Even though some of the results are negative, they are all close enough to consider ADIA well recovered. These values are like those reported previously by Christian, 2014; Bodine et al., 2002; and Kanani et al., 2014, who reported average recovery rates of 98.4%, 97%, and 103%, respectively. These data are important because while others reported good recovery of internal markers (Cochran et al., 1988; Bodine et al., 2002; Kanani et al., 2014), these recoveries were from diets where single forages were offered rather than multiple forages as were offered in the present study.

The differences in digestibility measurements calculated as actual DM digestibility, predicted using ADIA, were not different ($P \geq 0.93$) among treatments, indicating that the differences in forage proportions that resulted in nonlinear differences in digestibility of the diet did not impact the ability of ADIA to predict digestibility. Furthermore, the differences between actual and predicted dry matter digestibility were not different ($P \geq 0.51$) from zero, regardless of the forage combination and averaged -5.8 g/kg DM or 0.58 percentage units below DM digestibility determined by actual total fecal collections.

These data are important because, unlike other studies that evaluated the efficacy of using internal markers across different forage-based diets, this study evaluated the efficacy of ADIA in diets that had different combinations of forages of which one of the forages contained tannins that are known to suppress digestibility (Aguerre et al., 2016; Kronberg et al., 2018).

Table 2. Dry matter digestibility and recovery of acid detergent insoluble ash (ADIA) from ewe lambs offered diets of alfalfa silage alone or with different proportions of sericea lespedeza.

	Sericea lespedeza in diet, %[†]					
Item	**0**	**9**	**18**	**27**	**SEM[‡]**	***P*-value**
% of Dry Matter digestibility	68.0 a[§]	63.6 bc	64.4 b	60.9 c	1.16	0.0005
Acid Detergent Insoluble Ash recovery, g/d[¶]	-0.1	0.2	-0.4	-0.3	0.86	0.97
Actual-predicted digestibility, %[#]	-0.05	0.27	-1.21	-1.32	2.073	0.93

[†] The proportion (%) of sericea lespedeza in the diet dry matter. The remainder of the diet dry matter was alfalfa silage.

[‡] SEM = pooled standard error of the mean.

[§] Means within a row without a common letter differ ($P < 0.05$).

[¶] Calculated as the difference between acid detergent insoluble ash consumed (g/d) and that recovered in the feces (g/d).

[#] Calculated as the difference between actual dry matter digestibility (% dry matter) as determined by total fecal collection and dry matter digestibility as determined by the formula: 100- [100 x (Acid Detergent Insoluble Ash concentration in the feed/Acid Detergent Insoluble Ash concentration. in the feces)].

Conclusions

Acid detergent insoluble ash is a reliable tool that can be used in place of collecting total fecal output to determine diet digestibility. The importance of these results is that internal marker recovery for each group was the same over a range of diets, even those that contained components that impacted digestion negatively. Consistent internal marker recovery over a range of diets and species makes ADIA a reliable internal marker to determine total digestibility under a range of situations. Given that ADIA is faster and requires less labor, it proves to be a beneficial replacement to total fecal collections to determine total digestibility, which saves time and allows for greater numbers of observations in studies.

Acknowledgments

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