Journal of Animal and Feed Sciences, 12, 2003, 383-401

# Acid-insoluble ash as a marker in digestibility studies: a review

# J. Sales<sup>1</sup> and G.P.J. Janssens

Laboratory of Animal Nutrition, Department of Animal Nutrition, Genetics, Breeding and Ethology, Faculty of Veterinary Medicine, Ghent University Heidestraat 19, B-9820, Merelbeke, Belgium

(Received 23 January 2003; revised version 11 April 2003; accepted 15 July 2003)

#### Abstract

The use of markers to determine digestibility of feeds overcomes the need to make exact measurements of feed intake and total faecal output in the traditional total collection method. Although several external and internal markers have been evaluated through the years, a marker that satisfies all the criteria of an ideal marker is yet to be found. This review describes the use of acid-insoluble ash (AIA) as a marker in digestibility studies. Three variations of the original gravimetrically method, based on burning of organic matter in the sample by ashing, boiling in hydrochloric acid, and re-ashing, are commonly used to determine AIA contents. A summary of the recovery rate of AIA determined in several species with different diets presented a mean around 100 %. Of 45 studies where the AIA method was compared to the total collection method to determine digestibility of feeds in different species, 26 showed similar results, 9 an underestimation by the AIA method, and 10 an overestimation. No significant diurnal or daily variation in faecal AIA has been found in poultry, sheep, pigs, or cattle. Analytical error could be described as the most common reason for failure when using AIA as marker, especially in feeds with low natural AIA content. It is concluded that AIA presents a reliable marker with several advantages that could be successfully utilize to determine faecal digestibility in animal species under certain circumstances, and with the application of some precautions.

KEY WORDS: acid-insoluble ash, markers, digestibility, review

<sup>&</sup>lt;sup>1</sup> Corresponding author: e-mail: james.sales@rug.ac.be

#### INTRODUCTION

Reliable data on nutrient availability is required to determine nutrient requirements of animals, evaluate nutritive value of feed ingredients that present potential for dietary inclusion, develop least-cost feed formulations, and to minimize environmental impact of animal production (Vandenberg and Noüe, 2001). Measurement of total feed intake and faecal output is the widely used method to determine digestibility of feeds in animal species, but the reliability of the total collection method is debatable. Animals have to be housed in metabolism cages under unnatural conditions, that may not only influence their metabolism, but could also be difficult to justify from an animal welfare perspective. Furthermore, animals will be of a specific productive status, and, in practice accurate measurement of feed intake and precise collection of all faeces are not an easy and simple task. In poultry, where cages and trays placed under cages are the most common practice for total collection, problems, such as adherence of droppings to the birds' plumage, contamination of excreta with scurf and feathers, changes in chemical composition of excreta due to microbial activity, excreta losses during removal and transfer from trays to containers, birds excreting away from the tray, and droppings being contaminated with regurgitated feed, could complicate total collection of excreta. It is difficult to detect the above and even when observed, it is almost impossible to compensate for them in a meaningful way (McNab, 2000). The magnitude of these problems will increase enormously if large enclosures, due to welfare considerations, have to be used.

Certainly the use of markers to determine digestibility, as reviewed by Kotb and Luckey (1972) and Marais (2000), where measurement of feed intake and faecal output are eliminated, will be advantageous. Several external markers, such as chromium oxide, titanium oxide, rare earth elements, and internal markers, like indigestible acid detergent fibre, indigestible lignin, acid-insoluble ash (AIA) and *n*-alkanes, have been evaluated through the years in the search for a suitable marker. However, a marker that fulfils all the requirements of the ideal marker, namely, non-toxic, unaltered during its passage through the gut, no influence on the physiological processes in the digestive tract, closely associated to the undigested nutrient in question or flowing at an identical rate as the nutrient, and totally recovered in the faeces (Kotb and Luckey, 1972; De Silva, 1985; Marais, 2000), is yet to be found.

External markers must be mixed thoroughly with the feed or administrated to the animal, which may not be possible with large numbers of animals or animals under pasture conditions. Some feeds do not mix well with chemicals used as markers (Thonney et al., 1979; Rymer, 2000), and external markers might be excreted in a diurnal pattern, that precludes the use of grab faecal samples for determination of digestibility (Kotb and Luckey, 1972). Chromic oxide is probably the most

commonly used external marker in nutritional studies. However, poor repeatability (Vohra, 1972), and increasing awareness of hazards involved in analyses of chromic oxide (De Silva, 1989), are some of the reasons for the search of alternative markers, especially in fish digestibility studies (Austreng et al., 2000).

Among the internal markers, natural *n*-alkanes, if present at sufficient concentrations in the diet, seem promising in digestibility studies (Marais, 2000). However, *n*-alkanes are not fully recovered in faeces, thus recovery rate has to be determined and incorporated in the digestibility estimation. Furthermore, analyses of *n*-alkanes require the use of capillary gas chromatography (Dove and Mayes, 1991), a costly method. The most widely internal marker used in digestibility studies is AIA (Table 1), a substance that has also been used to determine feed intake

#### TABLE 1

Species	Nutrient	Reference	
Beef cattle	DM	Berger et al. (1981); Fulgoni (1984)	
		Barclay et al. (1986)	
		Schiere et al. (1989)	
		Huhtanen et al. (1994)	
	DM, OM, CP, energy,	Ayangbile et al. (1993)	
	DM, NDF, ADF	Staples et al. (1984)	
	DM, OM, CP, energy,		
	NDF, ADF, hemicellulose	Prado et al. (2001)	
Dairy cows	DM, OM, CP, energy NDF, ADF	Sharma et al. (1983)	
	hemicellulose	Udén (1984)	
	OM, CP, energy, starch	Pettersson and Martinsson (1994)	
Sheep	DM, energy, ADF, cellulose	Sharma et al. (1983)	
Alpine ibex	DM, OM, energy, CP fat, crude fibre, NFE,		
	NDF, ADF	Bassano et al. (1999)	
White rhinos	Energy	Frape et al. (1982)	
Giraffes	DM, CP, NDF, ADF	Clauss et al. (2001)	
Pigs	DM, energy, cell walls		
0	cellulose, hemicellulose	Varel et al. (1988)	
	DM, CP, fat	Qin et al. (2002)	
Horses	Energy	Frape et al. (1982)	
	DM, NDF, ADF	1 ( )	
	hemicellulose, cellulose OM, CP, energy	Glade and Sist (1988)	
	fat. NDF. ADF	Cuddeford et al. (1992)	

Studies where acid-insoluble ash had been used as marker to determine nutrient digestibility

#### TABLE 1

continue		
Species	Nutrient	Reference
Dogs	DM, energy, nitrogen	Johnson et al. (1998)
Cats	DM, CP, fat	Hesta et al. (2001)
Tortoises	Calcium, magnesium, phoshorus	Liesegang et al. (2001)
Broilers	Energy Amino acids Energy, amino acids	Schang et al. (1983); Scott and Baldaji (1997); Scott et al. (1998) Hew et al. (1998) Batal and Parsons (2002)
Ducks	Energy	Martin et al. (1998)
Ostrich	Energy	Farrell et al. (2001)
Emu	Energy	Farrell et al. (2001)
Trout	DM, CP DM, CP, energy, ash, lipid, NFE, phosphorus, magnesium,	Kabir et al. (1998)
	manganese, copper, iron, zinc Energy, fat, carbohydrate	Vandenberg and Noüe (2001)
	phosphorus, lysine	Rodehutschord et al. (2000, 2002)
Tilapia	DM, CP, energy CP, energy, fat	Goddard and McLean (2001) Sintayehu et al. (1996)
Nile catfish	CP, energy	Shabat (1993)
Prawn	СР	Deering et al. (1996)
Abalone	DM, OM, CP, energy, fat, starch	Sales and Britz (2001a,b; 2002)

in cattle (Cortada and Velloso, 1987; Bodine et al., 2000), faecal output in sheep (Santos and Petit, 1996), as well as a solid marker in studies on the partition of site of digestion in cattle (Miller et al., 1986; Kraiem et al., 1990) and horses (Almeida et al., 1998), and for determining ileal digestibility in ducks (Martin et al., 1998), poultry (Ravindran et al., 1999) and pigs (Fan and Sauer, 2002).

The aim of this review is to present information on the definition and determination of AIA, to make a relative comparison of digestibility values derived through this method to values obtained with the use of the total collection method, and to describe the advantages and disadvantages connected with its use as marker in digestibility studies.

#### DEFINITION AND DETERMINATION

Acid-insoluble ash consists of indigestible mineral components, mainly silica. Silica has been used as early as 1874 as digestibility indicator (Kotb and Luckey, 1972), but is not widely used as marker at present due to its possible absorption from the gut and the risk, and, of contamination of feed with soil, especially with grazing animals (Rymer, 2000). Acid-insoluble ash contents of feeds and faeces are determined gravimetrically after drying, ashing, boiling of ash in hydrochloric acid (HCl), filtering and washing of the hot hydrolysate, and re-ashing. Three variations to the original analytical method of Shrivastava and Talapatra (1962), consisting of the use of concentrated HCl, evaporation to dryness, and ashing at 650°C, are commonly in use. These are the adaptations by Vogtmann et al. (1975) in avian digestion studies, Van Keulen and Young (1977) in ruminants, pigs and horses, and Atkinson et al. (1984) in aquatic species (Table 2). In accordance to the method of Vogtmann et al. (1975), the ground sample has to be boiled in HCl before filtering and ashing. However, ashing of the sample prior to acid treatment removed the organic matter, thus reduces the acid concentration required (Van Keulen and Young, 1977). A higher ashing temperature to the method of Van Keulen and Young (1977) was introduced by Atkinson et al. (1984) to ensure complete combustion of the organic material in fish food with a high lipid content. Van Keulen and Young (1977) reported that, although dry matter digestibility of different diets were not significant different as from values determined with total collec-

Analytical conditions of methods to determine acid-insoluble ash in feed and faeces			
	Vogtmann et al. (1975)	Van Keulen and Young (1977)	Atkinson et al. (1984)
Feed, g	10-12	5	10-12
Faeces, g	5	5	3-4
HCl, N	4	2	2
HCL, ml	100	100	100
Boiling time, min	30	5	5
Ashing, °C	600	450 <sup>1</sup>	600 <sup>1</sup>
Ashing, h	minimum 4	overnight	16

<sup>1</sup>ashing before and after digestion in HCl

tion in sheep by either the methods of Vogtmann et al. or Van Keulen and Young, Vogtmann et al.'s method produced digestibility values that were significant higher than values determined according to the method of Van Keulen and Young. Mean recovery of AIA in faeces followed the same tendency, although none of the methods differed significantly from total (100 %) recovery. Significantly lower AIA contents were found with Vogtmann et al.'s method compared to that of Van Keulen and Young in feed samples.

TABLE 3

Acid-insoluble ash content of some feed ingredients (g/kg DM)			
Feed ingredient	AIA	Method	Reference
Ground maize	0.2	Van Keulen and Young (1977)	Thonney (1981)
Lucerne hay	3	Van Keulen and Young (1977)	Sunvold and Cochran (1991)
Lucerne hay			
high water-stressed	98	Van Keulen and Young (1977)	Undersander et al. (1987)
medium water-stressed	36	Van Keulen and Young (1977)	Undersander et al. (1987)
low water-stressed	37	Van Keulen and Young (1977)	Undersander et al. (1987)
Brome hay	21	Van Keulen and Young (1977)	Sunvold and Cochran (1991)
Prairie hay	45	Van Keulen and Young (1977)	Sunvold and Cochran (1991)
Fish meal	46	Atkinson et al. (1984)	Sales (unpublished results)
Soyabean meal	1	Atkinson et al. (1984)	Sales (unpublished results)
Cottonseed meal	2	Atkinson et al. (1984)	Sales (unpublished results)

Unrealistic digestibility values with the use of AIA as marker has been assigned to analytical imprecision due to a low dietary and faecal AIA content that introduced a large margin of error to the calculation (Van Keulen and Young, 1977; Jones and De Silva, 1998). Thonney et al. (1985) recommended that the dietary AIA content should exceed 7.5 g/kg on a dry matter (DM) basis in order to get accurate measurements and that appropriate diets should contain certain plant tissues that are high in AIA. The AIA content of some feed ingredients are presen-

In several studies the AIA content of feeds has been increased by addition of exogenous sources of AIA. Thus, acid-insoluble ash could also be classified as an external marker. The most frequently used exogenous sources of AIA is a diatomic product called Celite, but sand (Tillman and Waldroup, 1988a,b), acid-washed sand (Tacon and Rodrigues, 1984), volcanic ash (Thonney, 1981), silica (Cheng and Coon, 1990), Sipernat (Degussa AG, Frankfurt, Germany) (Rodehutscord et al., 1996) and bentonite (Sales and Britz, 2001a), have also been used. Although the addition of exogenous AIA sources will improve precision of measurement (Thonney, 1981), McCarthy et al. (1974) found that when Celite was added to pig diets, the AIA method underestimated digestibility of nitrogen and energy in com-

ted in Table 3.

parison to total collection, while comparable values were found between methods when Celite was excluded. They suggested that some Celite may be partly lost during feeding. A similar trend with addition of bentonite was reported in studies with abalone (Sales and Britz, 2001a). Tacon and Rodrigues (1984) found that precision decreased when dietary AIA concentration was increased to 20 g/kg with acid-washed sand in trout, while the opposite (increased precision) was found with the addition of a product of fine diatoms to pig diets (Jongbloed et al., 1991) and Celite to broiler diets (Scott and Baldaji, 1997).

Although Schang et al. (1983) stated that it is reasonable to assume that the flow of AIA through the alimentary tract of poultry reflects the passage of undigested feed residues, Wetherbee and Gruber (1993) reported differential intestinal passage rates for AIA and feed in the lemon shark. Also, Cheng and Coon (1990) postulated that, in the presence of a high dietary level of silica (> 0.2%), the intestinal flow of acid-insoluble ash might fail to reflect the passage of undigested feed. Thus, when adding ash or silica into the feed to enhance analytical accuracy of AIA, consideration should always be given to the possible inflation in the digestibility due to higher AIA intake (Cheng and Coon, 1990). Interference of added sand or other materials as primary source of AIA, instead of aiding, as intended, might happen. Determined AIA content in the feed might be lower than the actual value, or higher in faeces than was actually present (Tillman and Waldroup, 1988a).

#### RECOVERY RATE

Total faecal recovery, the most important criterion that must be met by any marker that is used in digestibility studies, is presented in Table 4 as the mean recovery rate of AIA determined with several diets.

Of 35 recovery rates presented in Table 4, 18 were within a five percentage unit range from 100 % recovery, and 25 within 10 percentage units. A recovery rate of 192 % was found when beef cattle were fed on lucerne hay, with a value of 101 % when fed prairie hay (Sunvold and Cochran, 1991). A similar variable recovery rate of 97 to 183 % were reported in pigs fed different maize based diets (Bakker and Jongbloed, 1994). The inconsistencies found in the above studies could be due to a low AIA content in feeds that will decrease analytical precision, soil ingestion by animals (Galyean et al., 1986), soil and dust in diets or faeces (Piaggio et al., 1991; Marais, 2000), or inaccurate feed sampling for AIA analysis. Environmental influences (water-stress) on lucerne had an influence on recovery rate of AIA found in sheep, leading to differences in dry matter, organic matter and cellulose digestibility coefficients between the total collection method and AIA marker method (Undersander et al., 1987). Except for three studies in Table 4 (Undersander et al., 1987; Piaggo et al., 1991; Zeoula et al. 1994), recovery rate of AIA was

Recovery rate of acid-insoluble ash with different species, %

Species	Diet	Recovery	Reference
Beef cattle	Concentrate, grass hay	100	Thonney et al. (1979)
	Roughage, concentrate	127	Wilson and Winter (1984)
	Lucerne hay	192	Sunvold and Cochran (1991)
	Brome hay	106	Sunvold and Cochran (1991)
	Prairie hay	101	Sunvold and Cochran (1991)
	Fescue hay	91	Bohnert et al. (2000)
Dairy cows	Silage, hay	119	Nishino et al. (1979)
	Нау		
	account for orts	99	Block et al. (1981)
	discard orts	118	Block et al. (1981)
	Hay, concentrate	100	Ohajuruka and Palmquist (1991)
Buffaloes	Urea-ensiled rice straw	105	Sriwattanasombat and Wanapat (1983)
Sheep	Lucerne, grains		
	2N HCl-method	96	Van Keulen and Young (1977)
	4N HCl method	103	Van Keulen and Young (1977)
	Maize plants		
	account for orts	100	Block et al. (1981)
	discard orts	104	Block et al. (1981)
	Нау		
	account for orts	101	Block et al. (1981)
	discard orts	98	Block et al. (1981)
	Pelleted feed	99	Piccolo et al. (1986)
	Lucerne hay		
	high water-stressed	90	Undersander et al. (1987)
	medium water-stressed	97	Undersander et al. (1987)
	low water-stressed	117	Undersander et al. (1987)
	Lucerne hay	84	Piaggio et al. (1991)
	Soyabeans, oats hay		
	free intake	151	Zeoula et al. (1994)
	restricted intake	115	Zeoula et al. (1994)
Goats	Rice straw, concentrate	88	Trung et al. (1987)
Pigs	Barley based	93	Wünsche et al. (1984);
		91	Moughan et al. (1991)
	Maize, soya, apple pomace	97	Furuya et al. (2001)
	Cereal based	100	Kavanagh et al. (2001)
	Wheat middlings	91	Yin et al. (2001)
Horses	Grass, sugarcane	101	Pereira and Queiroz (1997)
	Grass, hay	101	Araújo et al. (2000)
Dogs	Maize, animal meal	96	Johnson et al. (1998)
Ostriches	Concentrate, lucerne	127	Nizza and Meo (2000)
Humans	Celite	92	Rowan et al. (1991)

around 100 % for all studies on sheep. The recovery rate of over 100 % in ostriches was probably attributed to their feeding habits of grit intake, thus accumulating siliceous particles in their proventriculum (Nizza and Meo, 2000). However, the method of Vogtmann et al. (1975), used in the latter study, resulted in higher recovery rates than the method of Van Keulen and Young (1977).

When feeding cattle and sheep *ad libitum*, the AIA method may be questionable unless feed offered and orts are weighed regularly and sampled for AIA con-

with total concetion	(10)		
Species	Nutrient	Compared with TC	Reference
Beef cattle	DM	Overestimate	Wilson and Winter (1984)
silage diet	DM	Similar	Thonney et al. (1985)
grain diet	DM	Underestimate	Thonney et al. (1985)
	OM	Similar	Sunvold and Cochran (1991)
Dairy cows	СР	Overestimate	Nishino et al. (1979)
	DM	Similar	Ohajuruka and Palmquist (1991)
Buffaloes	DM, CP, NDF	Similar	Sriwattanasombat and Wanapat (1983)
Bison	DM, CP, energy, crude fibre, NDF, ADF, hemicellulose, lignin	Overestimate	Hawley et al. (1981)
Sheep	DM	Similar	Van Keulen and Young (1977)
1	DM	Underestimate	Aguilar et al. (1983)
	DM	Similar	Thonney et al. (1985)
	OM	Underestimate	Penning and Johnson (1983)
	OM	Underestimate	Piaggio et al. (1991)
	DM, OM, CP		
	NDF, ADF	Similar	Cheva-Isarakul and Saengdee (1986)
	DM, nitrogen,		
	ADF, NDF	Similar	Santos and Petit (1996)
	СР	Similar	Nishino et al. (1979)
Goats	DM, OM, CP, energy, NDF, ADF, cellulose DM	Similar Underestimate	Carvalho et al. (1992) Resende et al. (1996)
	12111	Chaerestinate	10000100 01 01. (1990)

Digestibility values derived through acid-insoluble ash as marker in comparison to values obtained with total collection (TC)

# 391

continue			
Species	Nutrient	Compared with TC	Reference
Pigs	DM DM, OM, energy DM, OM, CP DM, CP	Similar Similar Similar Similar	Rowan et al. (1991) Moughan et al. (1991) Borgmann et al. (1985) Furuya et al. (2001)
Mong Cai	DM, OM, nitrogen, NDF DM, OM	Overestimate	Ly et al. (2002)
Large White	nitrogen, NDF OM, CP	Similar Overestimate	Ly et al. (2002) Bakker and Jongbloed (1994)
12 weeks 19 weeks	Nitrogen, energy Nitrogen, energy CP, amino acids	Similar Similar Underestimate Similar	Yen et al. (1983) Yen et al. (1983) Wiesemuller et al. (1981)
Horses	DM Nitrogen, energy DM OM CP	Similar Similar	Orton et al. (1985) Sutton et al. (1977)
	ADF, NFE DM, OM, CP, energy, crude fibre, fat, ADF,	Similar	Cuddeford and Hughes (1990)
	NDF, NFE	Similar	Miraglia et al. (1999)
Kowari	DM	Overestimate	Trouten-Redford et al. (1995)
Laying hens	Ca	Overestimate	Cheng and Coon (1990)
Broilers	Energy Fatty acids	Underestimate Similar	Vogtmann et al. (1975) Vogtmann et al. (1975)
	Energy, amino acids	Overestimate	Tillman and Waldroup (1988a;b)
Ducks	DM, nitrogen, energy, Ca, P	Underestimate	Farrell and Martin (1998)
Barbary partridge	OM, CP, energy	Overestimate	Moniello et al. (2001)
Ostriches	DM, OM, CP, energy, fat	Overestimate	Nizza and Meo (2000)
Dogs	DM, CP, energy, fat, NFE	Similar	Lôbo et al. (2001)
Humans	DM	Similar	Rowan et al. (1991)
Abalone	DM	Similar	Sales and Britz (2001a)

tent, a sufficiently large number of animals is used, and diets are adequately mixed to limit feed selection and sorting (Block et al., 1981). The importance of taking orts into account in determination of digestibility when using AIA as marker is clearly illustrated in Table 4 in that mean values were closer to 100 % recovery when taking orts into account than when disregarding orts. An overestimation of 118 % for recovery rate was found when disregarding orts in dairy cows fed on hay. Disregarding of orts led to large deviations from 100 % recovery when orts represented more than 10 % of feed offered, and were caused by higher AIA contents in orts than in feed offered (Block et al., 1981).

# COMPARISON OF THE ACID-INSOLUBLE ASH MARKER METHOD WITH THE TOTAL COLLECTION METHOD

In Table 5 digestibility values derived with the AIA method is compared to values obtained through the total collection method. This relative comparison is presented according to statistical differences between methods as obtained in the mentioned studies.

Of 45 studies summarized in Table 5, 26 presented similar results between methods, while in nine the AIA method underestimated and 10 overestimated digestibility compared to the total collection method. In horses, and most studies with pigs, similar results were found between values determined with either total collection or AIA as marker. McCarthy et al. (1977) and Yen et al. (1983) stated that overestimation by total collection in heavier and older pigs could have been the result of failure to achieve total faecal collection in big metabolism cages. Acid-insoluble ash has caused overestimations compared to total collection in most studies with avian species (laying hens, broilers, partridges, ostriches) presented in Table 5. A possible explanation could be the use of the method of Vogtman et al. (1975) to determine AIA in avian digestion studies, a method that, compared to other methods, is leading to higher digestibility values, as earlier described.

Diurnal and daily variations in faecal AIA content were insignificant in poultry (Vogtmann et al., 1975), sheep (Van Keulen and Young, 1977; Thonney et al., 1985), pigs (McCarthy et al. 1977), cattle (Thonney et al., 1985), and horses (Cuddeford and Hughes, 1990), making the use of grab sampling possible. When using grab sampling, especially with diets that have a low AIA content, samples must be obtained from a number of animals over several days (Thonney, 1981; Thonney et al., 1985). More variation in metabolizability of energy and digestibility of amino acids has been reported in growing chickens (Tillman and Waldroup, 1988a,b) when applying the AIA method compared to total collection. However, the opposite (less variation when using the AIA method) has been found in ducks (Farrell and Martin, 1998).

#### CONCLUSIONS AND RECOMMENDATIONS

Acid-insoluble ash is a marker that could be used in digestibility studies in animals as an alternative to the total collection method. More natural housing could be applied, that will not only give a more reliable measurements of the animals' productive status, but will also be in accordance to animal welfare considerations. The AIA method, in accordance to the use of the marker technique to determine digestibility, has the advantage that grab samples could be taken from animals, resulting in obtaining samples from free-living animals before any chemical changes in faeces could occur. Furthermore, animals that have a social structure could be kept in groups for digestibility studies.

Special care should be taken when applying this method to grazing animals and outdoor pigs where ingestion of soil could occur, or for avain species like ratites and pigeons, where ingestion of grit could led to accumulation of siliceous components in the digestive tract. Soil and dust in feed and laboratories should be eliminated. When using grab sampling, samples have to be taken from several animals on different days, especially when the AIA contents of diets are low, and the AIA contents of orts have to be accounted for. The use of substances to increase the AIA content of feeds must be considered with great care.

The use of AIA as marker in digestibility studies is easy and simple in that the method of determination is simple and does not involve costly equipment or chemicals, but difficult in that a high degree of precision and skill is needed during analysis for it. Most digestibility studies where AIA had not met the expectations of a suitable marker did not attribute this to marker failure, but to analytical inaccuracy due mainly to low AIA contents. Suggestions for further research would be to evaluate the different methods available to determine AIA across feeds, faeces, and laboratories, and standardized (sample size, ashing temperature, amount of wash water) it to one reliable method in order to get high repeatability.

#### REFERENCES

- Aguilar A., Chel L., Castellanos A., 1983. A comparative study of methods to determine the digestibility of feedstuffs for ruminants and monogastrics. Trop. Anim. Prod. 8, 72-73
- Almeida de F.Q., Campos Valadares Filho de S., Silva da J.F.C., Alvarenga R.C., Cecon P.R., Leão M., 1998. Ileal flow of dry matter obtained with external and internal markers in equines (in Portuguese). Rev. Soc. Bras. Zoot. 27, 513-520
- Araújo K.V., Lima J.A., Fialho E.T., Miyagi E.S., 2000. Comparison among the internal markers and the total collection method in the determination of the forage nutrient digestibilities in equines (in Portuguese). Rev. Soc. Bras. Zoot. 29, 745-751
- Atkinson J.L., Hilton J.W., Slinger S.J., 1984. Evaluation of acid-insoluble ash as an indicator of feed digestibility in rainbow trout (*Salmo gairdneri*). Can. J. Fisheries Aquat. Sci. 41, 1384-1386

#### 394

- Austreng E., Storebakken T., Thomassen M.S., Refstie S., Thomassen Y., 2000. Evaluation of selected trivalent metal oxides as inert markers used to estimate apparent digestibility in salmonids. Aquaculture 188, 65-78
- Ayangbile O.A., Tallam S.K., Surtan M.S., 1993. Processing of slaughterhouse blood and poultry litter and the effects on nutrient digestibility of steers. Anim. Feed Sci. Tech. 40, 153-164
- Bakker G.C.M., Jongbloed A.W., 1994. The effect of housing system on apparent digestibility in pigs, using the classical and marker (chromic oxide, acid-insoluble ash) techniques, in relation to dietary composition. J. Sci. Food Agr. 64, 107-115
- Barclay R.A., Faulkner D.B., Fahey G.C. Jr., Cmarik G.F., 1986. Effects of salinomycin on performance characteristics and apparent dry matter digestion by grazing beef steers. Nutr. Rep. Int. 33, 43-54
- Bassano B., Bergero D., Tarantola M., Martys M., 1999. Diet digestibility in captive Alpine ibex (Capra ibex ibex). Zoot. Nutr. Anim. 25, 43-47
- Batal A.B., Parsons C.M., 2002. Effects of age on nutrient digestibility in chicks fed different diets. Poultry Sci. 81, 400-407
- Berger L.L., Ricke S.C., Fahey G.C. Jr., 1981. Comparison of two forms and two levels of lasalocid with monensin on feedlot cattle performance. J. Anim. Sci. 53, 1440-1445
- Bergero D., Peiretti P.G., Cola E., 2002. Intake and apparent digestibility of perennial ryegrass haylages fed to ponies either at maintenance or at work. Livest. Prod. Sci. 77, 325-329
- Block E., Kilmer L.H., Muller L.D., 1981. Acid insoluble ash as a marker of digestibility for sheep fed corn plants or hay and for lactating dairy cattle fed hay *ad libitum*. J. Anim. Sci. 52, 1164-1169
- Bodine T.N., Purvis H.T. II, Cox, D.A., 2000. Effects of supplemental energy and degradable intake protein on grazing behavior, forage intake, digestion and performance of steers grazing winter range. Animal Science Research Report - Agricultural Experiment Station, Oklahoma State University, No. P-980 (USA), pp. 33-39
- Bohnert D.W., Harmon D.L., Larson B.T., 2000. Evaluation of four internal markers and an intra-ruminal chromium-releasing device for use in predicting diet digestibility and intake in beef steers. Kentucky Beef Cattle Research Report, Agricultural Experimental Station, College of Agriculture, University of Kentucky, KY (USA), pp. 29-30
- Borgmann E., Wünsche J., Hennig U., Kreienbring F., Bock H.D., 1985. Estimation of nutrient digestibility in pigs with HCl-insoluble ash as indicator (in German). Tierernähr. Futt. 14, 143-148
- Carvalho de F.F.R., Queiros de A.C., Rodrigues M.T., Fontes de C.A., 1992. Use of internal markers to estimate digestibility of nutrients for lactating goats fed *ad libitum* (in Portuguese). Rev. Soc. Brasil. Zoot. 21, 270-278
- Cheng T.K., Coon C.N., 1990. Research Note: Calcium digestibility studies utilizing acid-insoluble ash measurements. Poultry Sci. 69, 2228-2230
- Cheva-Isarakul B., Saengdee C., 1986. Voluntary intake, nutrient digestibility and nitrogen balance in sheep fed sorghum. Ruminant feeding systems utilizing fibrous agricultural residues - 1986, Proceedings of the 6th Annual Workshop of the Australian-Asian Fibrous Agricultural Residues Research Network, Los Baños, 1-3 April, International Development Program of Australian Universities and Colleges Ltd., Canberra (Australia), pp. 133-138
- Clauss M., Lechner-Doll M., Flach E.J., Tack C., Hatt J-M., 2001. Comparative use of four different marker systems for the estimation of digestibility and low food intake in a group of captive giraffes (*Giraffa camelopardalis*). Zoo Biol. 20, 315-329
- Cortada C.N.M., Velloso L., 1987. Acid insoluble ash used to determine dry matter intake by cattle (in Portuguese). Rev. Fac. Med. Vet. Zoot. Univ. Sao Paulo 24, 61-64
- Cuddeford D., Hughes D., 1990. A comparison between chromium-mordanted hay and acid-insoluble ash to determine apparent digestibility of a chaffed, molassed hay/straw mixture. Equine Vet. J. 22, 122-125

- Cuddeford D., Woodhead A., Muirhead R., 1992. A comparison between the nutritive value of short-cutting cycle, high temperature-dried alfalfa and timothy hay for horses. Equine Vet. J. 24, 84-89
- Deering M.J., Hewitt D.R., Sarac H.Z., 1996. A comparison of inert markers used to estimate protein digestibility in the leader prawn *Penaeus monodon*. J. World Aquacult. Soc. 27, 103-106
- De Silva S.S., 1985. Evaluation of the use of internal and external markers in digestibility studies. In: C.Y. Cho, C.B. Cowey, T. Watanabe (Editors), Finfish Nutrition in Asia. Methodological Approaches to Research and Development, International Development Research Centre, Ottawa (Canada), pp. 96-102
- De Silva S.S., 1989. Digestibility evaluations of natural and artificial diets. In: S.S. De Silva (Editor), Proceedings of the Third Asian Fish Nutrition Network Meeting, Fish Nutrition Research in Asia. Asian Fisheries Society, Special Publication 4, Manila (Philippines), pp. 36-45
- Dove H., Mayes R.W., 1991. The use of plant wax alkanes as marker substances in studies of the nutrition of herbivores: a review. Aust. J. Agr. Res. 42, 913-952
- Fan M.Z., Sauer W.C., 2002. Determination of true ileal amino acid digestibility and the endogenous amino acid outputs associated with barley samples for growing-finishing pigs by regression analysis technique. J. Anim. Sci. 80, 1593-1605
- Farrell D.J., Martin E.A., 1998. Strategies to improve the nutritive value of rice bran in poultry diets. III. The addition of inorganic phosphorus and a phytase to duck diets. Brit. Poultry Sci. 39, 601-611
- Farrell D.J., Sales J., Perez-Maldonado R., Kent P., Shermer M., Mannion P.F., 2001. The apparent metabolisable energy of diets with different sources of fibre when fed to emus, ostriches and cockerels. In: A. Chwalibog, K. Jakobsen (Editors). Proceedings of the 15<sup>th</sup> Symposium on Energy Metabolism in Animals, Copenhagen (Denmark). EAAP Publication No. 103, Wageningen Press, Wageningen, pp. 141-143
- Frape D.L., Tuck M.G., Sutcliffe N.H., Jones D.B., 1982. The use of inert markers in the measurement of the digestibility of cubed concentrates and of hay given in several proportions to the pony, horse and white rhinoceros (*Diceros simus*). Comp. Biochem. Physiol. 72A, 77-83
- Fulgoni V. L., 1984. The relationship of body size, lactation or gestation with ration intake, nutrient digestibility and digestible nutrient intake in beef cattle. Diss. Abstr. Int. B (Sci. Eng.) 45, 3-4
- Furuya S., Yamamoto A., Itoh M., Aoki Y., 2001. Use of acid-insoluble ash added with celite as a marker for determining digestibility in pigs (in Japanese). Jpn. J. Swine Res. 38, 171-176
- Galyean M.L., Krysl L.J., Estell R.E., 1986. Marker-based approaches for estimation of fecal output and digestibility in ruminants. In: F.N. Owens (Editor). Feed Intake by Beef Cattle. Symposium, Oklahoma Agricultural Experimental Station, MP-121, Stillwater (USA), pp. 96-113
- Glade M.J., Sist M.D., 1988. Dietary yeast culture supplementation enhances urea recycling in the equine large intestine. Nutr. Rep. Int. 37, 11-17
- Goddard J.S., McLean E., 2001. Acid-insoluble ash as an inert reference material for digestibility studies in tilapia, *Oreochromis aureus*. Aquaculture 194, 93-98
- Hatt J.-M., Gisler R., Mayes R.W., Lechner-Doll M., Clauss M., Liesegang A., Wanner M., 2002. The use of dosed and herbage N-alkanes as markers for the determination of intake, digestibility, mean retention time and diet selection in Galapagos tortoises (*Geochelone nigra*). Herpetol. J. 12, 45-54
- Hawley A.W.L., Peden D.G., Stricklin W.R., 1981. Bison and Hereford steer digestion of sedge hay. Can. J. Anim. Sci. 61, 165-174
- Hesta M., Janssens G.P.J., Debraekeleer J., De Wilde R., 2001. The effect of oligofructose and inulin on faecal characteristics and nutrient digestibility in healthy cats. J. Anim. Physiol. Anim. Nutr. 85, 135-141

- Hew L.I., Ravindran V., Mollah Y., Bryden W.L., 1998. Influence of exogenous xylanase supplementation on apparent metabolisable energy and amino acid digestibility in wheat for broilers. Anim. Feed Sci. Tech. 75, 83-92
- Huhtanen P., Kaustell K., Joakkola S., 1994. The use of internal markers to predict total digestibility and duodenal flow of nutrients in cattle given six diets. Anim. Feed Sci. Tech. 48, 211-227
- Johnson M.L., Parsons C.M., Fahey G.C. Jr., Merchen N.R., Aldrich C.G., 1998. Effects of species raw material source, ash content, and processing temperature on amino acid digestibility of animal by-product meals by cecectomized roosters and ileally cannulated dogs. J. Anim. Sci. 76, 1112-1122
- Jones P.L., De Silva S.S., 1998. Comparison of internal and external markers in digestibility studies involving the Australian freshwater crayfish, *Cherax destructor* Clark (Decapoda, Parastacidae). Aquac. Res. 29, 487-493
- Jongbloed A.W., Bakker J.G.M., Goedhart P.W., Krol-Kramer F., 1991. Evaluation of chromic oxide with lower concentration and of HCl-insoluble ash as markers for measuring overall apparent digestibility of some dietary nutrients for pigs. Proceedings of the 5th International Symposium on Digestive Physiology in Pigs, Wageningen, (The Netherlands), pp. 325-329
- Kabir N.M.J., Wee K.L., Maguire G., 1998. Estimation of apparent digestibility coefficients in rainbow trout (*Oncorhynchus mykiss*) using different markers. Aquaculture 167, 259-272
- Kavanagh S., Lynch P.B., O'Mara F., Caffrey P.J., 2001. A comparison of total collection and marker technique for the measurement of apparent digestibility of diets for growing pigs. Anim. Feed Sci. Tech. 89, 49-58
- Kotb A.R., Luckey T.D., 1972. Markers in nutrition. Nutr. Abstr. Rev. 42, 813-845
- Kraiem K., Garrett J.E., Meiske J.C., Goodrich R.D., Marten G.C., 1990. Influence of method of forage preservation on fibre and protein digestion in cattle given lucerne, birdsfoot trefoil and sainfoin. Anim. Prod. 50, 221-230
- Liesegang A., Hatt J-M., Nijboer J., Forrer R. Wanner M., Isenbügel E., 2001. Influence of different dietary calcium levels on the digestibility of Ca, Mg, and P in captive-born juvenile Galapagos giant tortoises (*Geochelone nigra*). Zoo Biol. 20, 367-374
- Lõbo Júnior M.F., Rezende A.S.C., Saliba E.O.S., Sampaio I.B.M., 2001. Determination of apparent digestibility coefficients either by markers or total fecal collection techniques in dogs (in Portuguese). Arq. Bras. Med. Vet. Zoot. 53, 691-694
- Ly J., Ty C., Samkol P., 2002. Studies on the use of acid insoluble ash as inert marker in digestibility trials with Mong Cai pigs. Lives. Res. Rural Develop. 14, Issue 5 (On-line edition)
- Marais J.P., 2000. Use of markers. In: J.P.F. Mello (Editor). Farm Animal Metabolism and Nutrition. CABI International, Wallingford, Oxon (UK), pp. 255-277
- Martin E.A., Nolan J.V., Nitsan Z., Farrell D.J., 1998. Strategies to improve the nutritive value of rice bran in poultry diets. IV. Effects of addition of fish meal and a microbial phytase to duckling diets on bird performance and amino acid digestibility. Brit. Poultry Sci. 39, 612-621
- McCarthy, J.F., Aherne, F.X., Okai, D.B., 1974. Use of HCl insoluble ash as an index material for determining apparent digestibility with pigs. Can. J. Anim. Sci. 54, 107-109
- McCarthy J.F., Bowland J.P., Aherne F.X., 1977. Influence of method upon the determination of apparent digestibility in the pig. Can. J. Anim. Sci. 57, 131-135
- McNab J.M., 2000. Rapid metabolizable energy assays. In: J.P.F. Mello (Editor). Farm Animal Metabolism and Nutrition. CABI International, Wallingford, Oxon (UK), pp. 307-315
- Miller B.L., Meiske J.C., Goodrich R.D., 1986. Effects of dietary additives on B-vitamin production and absorption in steers. J. Anim. Sci. 62, 484-496
- Miraglia N., Bergero D., Bassano B., Tarantola M., Ladetto G., 1999. Studies of apparent digestibility in horses and the use of internal markers. Livest. Prod. Sci. 60, 21-25

- Moniello G., Pinna W., Nizza A., Stanco G., Solinas I.L., 2001. Digestion capabilities and estimation of metabolisable energy of diets in relation to their chemical components in Barbary partridge (*Alectoris Barbara*) (in Italian). Riv. Avicolt. 70, 37-40
- Moughan P.J., Smith W.C., Schrama J., Smits C., 1991. Chromic oxide and acid-insoluble ash as faecal markers in digestibility studies with young growing pigs. N.Z. J. Agr. Res. 34, 85-88
- Nishino S., Kondo S., Ogino K., 1979. Determinations on acid-insoluble ash as index substance of digestion trials with sheep and lactating cows (in Japanese). J. Coll. Dairying 8, 23-30
- Nizza A., Meo de C., 2000. Determination of apparent digestibility coefficients in 6-, 12-and 18week-old ostriches. Brit. Poultry Sci. 41, 518-520
- Ohajuruku O.A., Palmquist D.L., 1991. Evaluation of *n*-alkanes as digesta markers in dairy cows. J. Anim. Sci. 69, 1726-1732
- Orton R.K., Hume I.D., Leng R.A., 1985. Effects of exercise and level of dietary protein on digestive function in horses. Equine Vet. J. 17, 386-390
- Penning P.D., Johnson R.H., 1983. The use of internal markers to estimate herbage digestibility and intake. 1. Potentially indigestible cellulose and acid insoluble ash. J. Agr. Sci. 100, 127-131
- Pereira J.C., Queiroz de A.C., 1997. Apparent digestibility in horses fed different proportions of elephant grass (*Pennisetum purpureum*, Schum) and sugarcane (*Succharum officinarum*, L.) (in Portuguese). Rev. Soc. Bras. Zoot. 26, 105-110
- Pettersson T., Martinsson K.A., 1994. Digestibility of whole or rolled ensiled barley grain fed to heifers or lactating cows. Swed. J. Agr. Res. 24, 109-113
- Piaggio L.M., Pratas Ê.R., Pires F.F., Ospina H., 1991. Evaluation of acid insoluble ash, nondigestible acid detergent fibre and nondigestible acid detergent lignin as internal markers of digestibility (in Portuguese). Rev. Soc. Bras. Zoot. 20, 306-312
- Piccolo V., Nizza A., Lella T., 1986. Observations on different methods of estimating apparent digestibility coefficients in wethers (in Italian). Zoot. Nutr. Anim. 12, 295-299
- Prado I.N., Zeoula L.M., Vinocur K., Medroni S., Nascimento W.G., 2001. Reduction of bromatological analyses in feed intake, feed conversion and apparent digestibility for feedlot heifers (in Portuguese). Acta Scient. 23, 1069-1073
- Qin G.X., Xu L.M., Jiang H.L., Poel van der A.F.B., Bosch M.W., Verstegen M.W.A., 2002. The effects of Chinese and Argentine soyabeans on nutrient digestibility and organ morphology in Landrace and Chinese Min pigs. Asian-Austr.) J. Anim. Sci. 15, 555-564
- Ravindran V., Hew L.I., Ravindran G., Bryden W.L., 1999. A comparison of ileal digesta and excreta analysis for the determination of amino acid digestibility in food ingredients for poultry. Brit Poultry Sci. 40, 266-274
- Resende de K.T., Furlan C.L., Costa R.G., Sugohara A., Vascondelos V.R., Carvalho de F.F.R., Guideli C., 1996. Use of chromated collagen as an indicator in digestion studies in goats (in Portuguese). Rev. Soc. Bras. Zoot. 25, 806-813
- Rodehutscord M., Borchert F., Gregus Z., Pfeffer E., 2002. Availability and utilisation of free lysine in rainbow trout (*Oncorhynchus mykiss*). 2. Comparison of L-lysine-HCl and L-lysine sulphate. Aquaculture 187, 177-183
- Rodehutscord M., Faust M., Lorenz H., 1996. Digestibility of phosphorus contained in soybean meal, barley, and different varieties of wheat, without and with supplemental phytase fed to pigs and additivity of digestibility in a wheat-soy-bean-meal diet. J. Anim. Physiol. Anim. Nutr. 75, 40-48
- Rodehutscord M., Gregus Z., Pfeffer E., 2000. Effect of phosphorus intake on faecal and non-faecal phosphorus excretion in rainbow trout (*Oncorhynchus mykiss*) and the consequences for comparitive phosphorus availability. Aquaculture 188, 383-398
- Rowan A.M., Moughan P.J., Wilson M.N., 1991. Acid-insoluble ash as a marker compound for use in digestibility studies with humans. J. Sci. Food Agr. 54, 269-274

- Rymer C., 2000. The measurement of forage digestibility *in vivo*. In: D.I. Givens, E. Owen, R.F.E. Axford, H.M. Ohmed (Editors). Forage Evaluation in Ruminants. CABI International, Wallingford, Oxon (UK), pp. 113-132
- Sales J., Britz P.J., 2001a. Evaluation of the reference diet substitution method for determination of apparent nutrient digestibility coefficients of feed ingredients for South African abalone (*Ha-liotis midae* L.). Aquaculture 207, 113-123
- Sales J., Britz P.J., 2001b. Evaluation of different markers to determine apparent nutrient digestibility coefficients of feed ingredients for South African abalone, (*Haliotis midae* L.). Aquaculture 202, 113-129
- Sales J., Britz P.J., 2002. Influence of ingredient particle size and dietary inclusion level of pre-gelatinised maize starch on apparent nutrient digestibility of diets for South African abalone (*Haliotis midae* L.). Aquaculture 212, 299-309
- Santos dos G.T., Petit H.V., 1996. Prediction of total fecal output in sheep fed silage using the Captec chrome controlled-release capsule. Small Ruminant Res. 20, 223-227
- Schang M.J., Sibbald I.R., Hamilton R.M.G., 1983. Comparison of two direct bioassays using young chicks and two internal indicators for estimating the metabolizable energy content of feedingstuffs. Poultry Sci. 62, 117-124
- Schiere J.B., Ibrahim M.N.M., Sewalt V.J.H., Zemmelink G., 1989. Response of growing cattle given rice straw to lickblocks containing urea and molasses. Anim. Feed Sci. Tech. 26, 179-189
- Scott T.A., Baldaji F., 1997. Comparison of inert markers [chromic oxide or insoluble ash (Celite™)] for determining apparent metabolizable energy of wheat-or barley-based broiler diets with or without enzymes. Poultry Sci. 76, 594-598
- Scott T.A., Silversides F.G., Classen H.L., Swift M.L., Bedford M.R., Hall J.W., 1998. A broiler chick bioassay for measuring the feeding value of wheat and barley in complete diets. Poultry Sci. 77, 449-455
- Shabat T.M., 1993. Digestibility determination in Nile catfish fingerlings using internal and external markers. Vet. Med. J. Giza 41, 83-91
- Sharma H.R., Ingalls J.R., McKirdy J.A., 1983. Feeding value of alkali-treated whole rye (*Secale cereale* L.) grain for lactating cows and its digestibility for sheep. Anim. Feed Sci. Tech. 10, 77-82
- Shrivastava V.S., Talapatra S.K., 1962. Pasture studies in Uttar Pradesh. II. Use of some natural indicators to determine the plane of nutrition of a grazing animal. Indian J. Dairy Sci. 15, 154-160
- Sintayehu A., Mathies E., Meyer-Burgdorf K.H., Rosenow H., Günther K.D., 1996. Apparent digestibilities and growth experiments with tilapia (*Oreochromis nilocitus*) fed soybean meal, cottonseed meal and sunflower seed meal. J. Appl. Ichthyol. 12, 125-130
- Sriwattanasombat P., Wanapat M., 1983. Supplementation of urea-treated straw with dried leucaena (*Leucaena leucocephalia*) and water hyacinth (*Eichhornia crassipes*) leaf meals. Annual Report, The National Buffalo Research and Development Center Project, Department of Livestock Development, Bangkok (Thailand), pp. 170-179
- Staples C.R., Fernando R.L., Fahey G.C. Jr., Berger L.L., Jaster E.H., 1984. Effects of intake of a mixed diet by dairy steers on digestion events. J. Dairy Sci. 67, 995-1006
- Sunvold G.D., Cochran R.C., 1991. Technical note: evaluation of acid detergent lignin, alkaline peroxide lignin, acid insoluble ash, and indigestible acid detergent fiber as internal markers for prediction of alfalfa, bromegrass, and prairie hay digestibility by beef steers. J. Anim. Sci. 69, 4951-4955
- Sutton E.I., Bowland J.P., McCarthy J. F., 1977. Studies with horses comparing 4N-HCl insoluble ash as an index material with total fecal collection in the determination of apparent digestibilities. Can. J. Anim. Sci. 57, 543-549
- Tacon A.G.J., Rodrigues A.M.P., 1984. Comparison of chromic oxide, crude fibre, polyethylene and acid-insoluble ash as dietary markers for the estimation of apparent digestibility coefficients in rainbow trout. Aquaculture 43, 391-399

- Thonney M.L., 1981. Acid insoluble ash as a digestion marker. Proceedings of the Cornell Nutrition Conference, Cornell University, Ithaca, NY (USA), pp.118-122
- Thonney M.L., Duhaime D.J., Moe P.W., Reid J.T., 1979. Acid insoluble ash and permanganate lignin as indicators to determine digestibility of cattle rations. J. Anim. Sci. 49, 1112-1116
- Thonney M.L., Palhof B.A., DeCarlo M.R., Ross D.A., Firth N.L., Quaas R.L., Perosio D.J., Duhaime D.J., Rolins S.R., Nour A.Y.M., 1985. Sources of variation of dry matter digestibility measured by the acid insoluble ash marker. J. Dairy Sci. 68, 661-668
- Tillman P.B., Waldroup P.W., 1988a. Assessment of extruded grain amaranth as a feed ingredient for broilers. 1. Apparent metabolizable energy values. Poultry Sci. 67, 641-646
- Tillman P.B., Waldroup P.W., 1988b. Assessment of extruded grain amaranth as a feed ingredient for broilers. 2. Apparent amino acid availability values. Poultry Sci. 67, 647-651
- Trouten-Radford L.M., Valdes E.V., Dufresne A.L., Mcbride B.W., 1995. Total fecal collection and acid-insoluble ash as measures of dry matter digestibility by Kowari (*Dasyuriodes brynei*). Proceedings of the 1ste Annual Conference of the Nutrition Advisory Group of the American Zoo and Aquarium Association, Toronto (Canada), pp. 218-222
- Trung L.T., Palo L.P., Atega T.A., Bien R.R., Lapinid R.R., 1987. Dose responses of goats fed ureatreated rice straw with varying supplementation rates: intake and digestibility by markers. Ruminant feeding systems utilizing fibrous agricultural residues – 1987, International Development Program of Australian Universities and Colleges Ltd., Canberra (Australia), pp. 245-251
- Udén P., 1984. Digestibility and digesta retention in dairy cows receiving hay or silage at varying concentrate levels. Anim. Feed Sci. Tech. 11, 279-291
- Undersander D.J., Cole N.A., Naylor C.H., 1987. Digestibility by lambs of water-stressed alfalfa as determined by total collection or internal markers. J. Dairy Sci. 70, 1719-1723
- Vandenberg G.W., Noüe de la J., 2001. Apparent digestibility comparison in rainbow trout (Oncorhynchus mykiss) assessed using three methods of faeces collection and three digestibility markers. Aquacult. Nutr. 7, 237-245
- Van Keulen J., Young B.A., 1977. Evaluation of acid-insoluble ash as a natural marker in ruminant digestibility studies. J. Anim. Sci. 44, 282-287
- Varel V.H., Jung H.G., Pond W.G., 1988. Effects of dietary fiber on young adult genetically lean, obese and contemporary pigs: rate of passage, digestibility and microbiological data. J. Anim. Sci. 66, 707-712
- Vogtmann H., Pfirter H.P., Prabucki A.L., 1975. A new method of determining metabolizability of energy and digestibility of fatty acids in broiler diets. Brit. Poultry Sci. 16, 531-534
- Vohra P., 1972. Evaluation of metabolizable energy for poultry. World Poultry Sci. J. 28, 204-214
- Wetherbee B.M., Gruber S.H., 1993. Use of acid-insoluble ash as a marker in absorption efficiency studies with the lemon shark. Prog. Fish-Cult. 55, 270-274
- Wiesemuller W., Poppe S., Breite L., Meier H., 1981. Digestibility of amino acids in lucerne silages (in German). Tierernähr. Futt. 12, 277-288
- Wilson R.K., Winter K.A., 1984. An evaluation of acid-insoluble ash as a predictor of feed digestibility in ruminants. Irish J. Agr. Res. 23, 97-98
- Wünsche J., Borgmann E., Hennig U., Kreienbring F., Bock H.D., 1984. Use of ash insoluble in HCl as indicator for estimating digestibility of nutrients including amino acids at the end of the small intestine and of the whole digestive tract in pigs (in German). Arch. Anim. Nutr. 34, 817-831
- Yen J.T., Tess M.W., Pond W.G., Dickerson G.E., 1983. Digestibility and metabolism of dietary nitrogen and energy in contemporary, genetically lean and obese pigs as estimated by total fecal collection and acid insoluble ash. J. Anim. Sci. 56, 426-430

- Yin Y.-L., McEvoy J.D., Schulze H., McCracken K.J., 2001. Effects of xylanase and antibiotic addition on ileal and faecal apparent digestibilities of dietary nutrients and evaluating HCl-insoluble ash as a dietary marker in growing pigs. Anim. Sci. 72, 95-103
- Zeoula L.M., Borges I., Branco A.F., Prado I.N., Moreira H.L.M., Salina J.L., Moraes de G.V., 1994. Evaluation of markers in digestibility studies in ruminants (in Portuguese). Rev. Unimar 16, 165-174

#### STRESZCZENIE

#### Popiół nierozpuszczalny w kwasie jako wskaźnik w doświadczeniach nad oznaczaniem strawności składników pokarmowych: przegląd

Zastosowanie wskaźników do oznaczania strawności składników pokarmowych pasz pozwala na uniknięcie konieczności oznaczania ilości pobranej paszy i wydalonego kału. Choć w ciągu wielu lat badano wiele wewnętrznych i zewnętrznych wskaźników, to nie został jeszcze znaleziony taki, który spełniałby kryteria idealnego wskaźnika. W prezentowanym przeglądzie omówiono użycie popiołu nierozpuszczalnego w kwasie (AIA) jako wskaźnika w badaniach strawnościowych. Do oznaczania zawartości AIA stosowane są zwykle trzy warianty oryginalnej grawimetrycznej metody, opartej na spopieleniu badanych prób, gotowaniu w roztworze kwasu chlorowodorowego i powtórnym spopieleniu.

W podsumowaniu otrzymanych wyników w doświadczeniach przeprowadzonych na wielu gatunkach zwierząt żywionych różnymi paszami można uznać, że w kale znajduje się około 100% AIA podanego w dawce pokarmowej. W 44 doświadczeniach strawnościowych, w których porównano metodę wskaźnikową, z użyciem AIA, z wynikami metody klasycznej, wykonanych na różnych gatunkach zwierząt, w 26 otrzymano podobne wyniki, w 9 - były niższe, a w 10 wyższe. Nie stwierdzono istotnej dziennej zmienności w ilości AIA w kale drobiu, owiec, świń i bydła. Najpowszechniejszą przyczyną niepowodzenia przy zastosowaniu AIA jako wskaźnika są błędy analityczne, szczególnie w przypadku pasz o małej zawartości AIA.

W podsumowaniu można stwierdzić, że AIA jest dobrym wskaźnikiem, z wieloma zaletami, który może być z powodzeniem stosowany do oznaczania strawności składników pokarmowych w określonych warunkach i z zachowaniem pewnej ostrożności.