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

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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

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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) |
|                | DM, OM, CP, energy,       | Barclay et al. (1986)         |
|                | DM, NDF, ADF              | Schiere et al. (1989)         |
|                | DM, OM, CP, energy,       | Ayangbile et al. (1993)      |
|                | NDF, ADF, hemicellulose   | Staples et al. (1984)         |
| **Dairy cows**| DM, OM, CP, energy,       | Sharma et al. (1983)          |
|                | NDF, ADF                  | Udén (1984)                  |
|                | hemicellulose             | Pettersson and Martinsson (1994) |
|                | OM, CP, energy, starch    |                               |
| **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    | Varel et al. (1988)          |
|                | cellulose, hemicellulose  | Qin et al. (2002)            |
|                | DM, CP, fat               | Glade and Sist (1988)        |
| **Horses**     | Energy                    | Frape et al. (1982)          |
|                | DM, NDF, ADF              |                               |
|                | hemicellulose, cellulose  | Cuddeford et al. (1992)      |
|                | OM, CP, energy            |                               |
|                | fat, NDF, ADF             |                               |
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

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

| 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°            | 600°            |
| Ashing, h                      | minimum 4       | overnight       | 16              |

1ashing 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.

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 presented in Table 3.

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-

| Feed ingredient     | AIA  | Method               | Reference                      |
|---------------------|------|----------------------|--------------------------------|
| Ground maize        | 0.2  | Van Keulen and Young | Thonney (1981)                 |
| Lucerne hay         | 3    | Van Keulen and Young | Sunvold and Cochran (1991)     |
| Lucerne hay         |      |                      |                                |
| high water-stressed | 98   | Van Keulen and Young | Undersander et al. (1987)      |
| medium water-stressed| 36  | Van Keulen and Young | Undersander et al. (1987)      |
| low water-stressed  | 37   | Van Keulen and Young | Undersander et al. (1987)      |
| Brome hay           | 21   | Van Keulen and Young | Sunvold and Cochran (1991)     |
| Prairie hay         | 45   | Van Keulen and Young | 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)    |
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 (Galvey 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; Piaggio et al., 1991; Zeoula et al. 1994), recovery rate of AIA was
### TABLE 4

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)                         |
|             | Hay                                |          |                                               |
|             | 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)                           |
|             | Hay                                |          |                                               |
|             | 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)                         |
|             | Soyabean, 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);                        |
|             |                                   |          | 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-

| TABLE 5 | Digestibility values derived through acid-insoluble ash as marker in comparison to values obtained with total collection (TC) |
|---------|--------------------------------------------------------------------------------------------------------------------------|
| Species | Nutrient | Compared with TC | Reference |
| Beef cattle silage diet | DM | Overestimate | Wilson and Winter (1984) |
| grain diet | DM | Similar | Thonney et al. (1985) |
|          | OM | Underestimate | Thonney et al. (1985) |
| Dairy cows | CP | 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) |
|        | DM | Underestimate | Aguilar et al. (1983) |
|        | OM | Similar | Thonney et al. (1985) |
|        | OM | Underestimate | Penning and Johnson (1983) |
|        | DM, OM, CP NDF, ADF | Similar | Piaggio et al. (1991) |
|        | DM, nitrogen, ADF, NDF | Similar | Cheva-Isarakul and Saengdee (1986) |
|        | CP | Similar | Santos and Petit (1996) |
| Goats | DM, OM, CP, energy, NDF, ADF, cellulose | Similar | Nishino et al. (1979) |
|        | DM | Underestimate | Resende et al. (1992) |
| Species       | Nutrient                      | Compared with TC | Reference               |
|---------------|-------------------------------|------------------|-------------------------|
| Pigs          | DM                            | Similar          | Rowan et al. (1991)     |
|               | DM, OM, energy                | Similar          | Moughan et al. (1991)   |
|               | DM, OM, CP                    | Similar          | Borgmann et al. (1985)  |
|               | DM, CP                        | Similar          | Furuya et al. (2001)    |
| Mong Cai      | DM, OM, nitrogen, NDF         | Overestimate     | Ly et al. (2002)        |
| Large White   | DM, OM, nitrogen, NDF         | Similar          | Ly et al. (2002)        |
|               | OM, CP                        | Overestimate     | Bakker and Jongbloed (1994) |
|               | Energy                        | Similar          | Kavanagh et al. (2001)  |
| 12 weeks      | Nitrogen, energy              | Similar          | Yen et al. (1983)       |
| 19 weeks      | Nitrogen, energy              | Underestimate    | Yen et al. (1983)       |
|               | CP, amino acids               | Similar          | Wiesemuller et al. (1981) |
| Horses        | DM                            | Similar          | Orton et al. (1985)     |
|               | Nitrogen, energy              | Similar          | Sutton et al. (1977)    |
|               | DM, OM, CP, ADF, NFE          | Similar          | Cuddeford and Hughes (1990) |
|               | DM, OM, CP, energy, crude fibre, fat, ADF, 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                        | Underestimate    | Vogtmann et al. (1975)  |
|               | Fatty acids                   | Similar          | 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          | Lóbo 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 Vogtmann 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 avian 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.

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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 powtórny spopieleniu.

W podsumowaniu otrzymanych wyników w doświadczeniach przeprowadzonych na wielu gatunkach zwierząt żywionych różnymi paszami można uznać, że w kału znajduje się około 100% AIA podanego w dawce pokarmowej. W 44 doświadczeniach strawnościowych, w których porównano metodę wskaźnikową, z użyciem AIA, z wynikami metody klasyjnej, 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 kału drobiu, owiec, świń i bydła. Najpowiesze wierzchnią 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.