Animal Feed Science and Technology 188 (2014) 92–101

Contents lists available at ScienceDirect

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci

Use of acid-insoluble ash and *n*-alkanes as markers of soil and plant ingestion by chickens

S. Jurjanz [a,*], K. Germain [b], M.A. Dziurla [a], H. Juin [b], C. Jondreville [a]

[a] INRA-Université de Lorraine, UR AFPA, 2 avenue de la forêt de Haye, TSA 40602, Vandoeuvre-lès-Nancy Cedex F-54518, France
[b] INRA, UE EASM, UE1206, Domaine du Magneraud, BP 52, Saint-Pierre-d'Amilly F-17700, France

ARTICLE INFO

Article history:
Received 26 October 2012
Received in revised form 4 November 2013
Accepted 8 November 2013

Keywords:
Chicken
Markers
Digestibility
Soil
Plants

ABSTRACT

Soil ingestion is recognized as the main source of environmental contaminants in food-producing animals reared outdoors. Therefore, its consumption should be quantified for proper risk assessment under practical circumstances. A quantitative method, involving the determination of acid-insoluble ash (AIA) in feces, was previously evaluated in several animal species. Nevertheless, this method is highly sensitive to feed digestibility, which may be influenced by the unknown amount of soil actually consumed. Besides, animals may ingest plants in addition to soil. Therefore the amount and the digestibility of plants ingested should also be known. The purpose of the current study was to assess a method for determining soil and plants ingested by free range chickens using AIA and odd-numbered *n*-alkanes (C25 to C31) as markers of soil and of plants, respectively. Ninety slow-growing chickens aged 38 days were placed in individual cages and given nine pelleted diets containing, in addition to standard feed, graded levels of soil and plants alone, or in combination (0 to 30% and 0 to 15% of diet dry matter (DM), respectively). After a 5-day adaptation period, excreta from each bird were collected in their entirety for three days. The energy retention coefficient (ERC) of feed alone and of plants was estimated to be 0.70 and 0.14, respectively. The presence of soil linearly depressed feed ERC but did not influence plant ERC. The effect of soil on feed ERC was quite low, reaching 0.02 for 30% of soil incorporation in diet DM. The average recovery rate of ingested *n*-alkanes in excreta was low (43%) and variable between *n*-alkanes and between diets, while AIA was totally recovered in all diets except in feed alone. After the concentration of each *n*-alkane in excreta was corrected by its mean recovery rate, comparison of the feed, plant and excreta profiles elicited an estimate of plant contribution to DM ingested biased by 0 to 2% of plant contribution to DM. Despite these biases and the slight influence of soil on feed ERC, introducing the ERC of feed alone into the soil-ingestion equation provided a good estimate of soil contribution to DM ingested. The sequential current methodology is a promising tool for evaluating soil ingestion by broilers reared outdoors under practical circumstances. However, further work is required to alleviate uncertainties concerning the impact of different types of soil on the ERC of feed given to animals.

Abbreviations: AIA, acid-insoluble ash; ASE, accelerated solvent extraction; DM, dry matter, Eprovided energy; Ef, energy provided by feed; Ep, energy provided by plants; ER, energy retention; GE, gross energy; GEf, gross energy provided by feed; GEp, gross energy provided by plants; LOQ, limit of quantification.

* Corresponding author at: UR AFPA, Université de Lorraine, 2 avenue de la forêt-de-Haye, TSA 40602, 54518 Vandoeuvre, France. Tel.: +33 383595832; fax: +33383595889.
E-mail address: stefan.jurjanz@univ-lorraine.fr (S. Jurjanz).

0377-8401/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.anifeedsci.2013.11.004

1. Introduction

In alternative rearing systems, the runs provided to animals would allow them to express their innate behavior to forage for their feed outdoors. Besides considerations of animal welfare, these rearing systems may allow a reduction in feed costs and the control of risk elements (N, P, metallic trace elements), thanks to the full use of local resources found on the runs (Horsted and Hermansen, 2007). However, surveys carried out in different European countries pointed to possible contamination by environmental contaminants (heavy metals such as lead, polychlorobiphenyls, dioxins-furans, and organochlorinated pesticides) of products from chickens granted outside access (Schoeters and Hoogenboom, 2006; De Vries et al., 2006; van Overmeire et al., 2009). Ingesting soil has been put forth as the main source of contaminants coming from the environment (Schuler et al., 1997; Waegeneers et al., 2009). According to Stephens et al. (1995) and De Vries et al. (2006), soil ingestion by laying hens would be circa 10 g per day. However, based on actual dioxin concentration in eggs, Waegeneers et al. (2009) estimated that it may range between 2 and 30 g daily. Knowledge of the amount of soil ingested by food-producing animals reared outdoors is thus a prerequisite for a relevant risk assessment, either in case of sanitary crises or in order to evaluate rearing practices that are likely to modulate soil ingestion.

Under practical circumstances, soil ingestion by animals may be estimated from the measurement of acid-insoluble ash in feces. This method, which requires knowledge of the amount of feed ingested and its digestibility, was used to estimate soil ingestion by pigs (Fries et al., 1982a), wildlife including birds (Beyer et al., 1994) and cattle (Healy, 1968; Fries et al., 1982b; Jurjanz et al., 2012). According to these authors, the digestibility of the feed ingested strongly impacts estimates of soil ingestion. Digestibility of feed provided to chickens is well-documented, but may be modified when ingested together with soil. In laying hens, van der Meulen et al. (2008) observed a 6- to 9-unit increase in DM digestibility of a layer feed when mixed with 20 to 30% sand, leading to a bias in the estimate of sand ingestion by means of the aforementioned method.

In addition to soil, chickens may ingest other items present in the run, particularly plants. Unlike feed, the amount of plants ingested by free-range chickens cannot be controlled under practical circumstances and may be subject to variations (Rivera Ferre et al., 2001; Horsted et al., 2006). Nevertheless, the amount and the digestibility of plants ingested should be known for proper determination of soil ingestion. The sward cutting method has been used to estimate herbage intake in free-range chickens (Rivera Ferre et al., 2001; Horsted et al., 2006). This method requires the isolation of plots within the run and may be cumbersome for use on commercial flocks. Alternatively, plant intake has been determined by using *n*-alkanes as markers in ruminants (Dove and Mayes, 1991) and in pigs (Rivera Ferre et al., 2001). Hameleers et al. (1996) measured the recovery of ingested *n*-alkanes in excreta of chickens and suggested that these compounds could be valuable markers for determining herbage intake by chickens in free-range situations.

The purpose of the current study was to evaluate a method for determining soil and plants ingested by free-range chickens using acid-insoluble ash and *n*-alkanes as markers. With this aim, chickens were fed diets made up of feed mixed with graded levels of soil and plants. The collection of excreta was used to assess the impact of soil on the energy value of the feed and plants, the effectiveness of *n*-alkanes as markers of plant intake, and finally the accuracy of acid-insoluble ash for estimating soil ingestion.

2. Materials and methods

2.1. Ingredients and experimental diets

A grower feed was purchased from a commercial mill (110.18 poussin croissance BIO®, Mercier Ltd, La-Roche-sur-Yon, France) and ground to pass through a 2 mm-sieve. Some top soil was sampled in the A-horizon of a tree-covered plot. After stones of sizes greater than 2 mm and plant fragments had been manually removed, the soil was dried for 10 days at room temperature before being sieved over a 2 mm screen. Plants were sampled in spring on a grassland plot which had been sown the year before. The main plant species were *Loliumperenne, Lotus corniculatus*, and *Trifoliumrepens*. Collected grass was washed to remove soil particles, dried (20 h at 60 °C) and ground to pass through a 2 mm-sieve.

Different mixtures of feed, soil, and plants were made up on a dry matter (DM) basis, leading to nine experimental diets. The control diet was made of feed only (diet 1). The experimental diets were obtained by incorporating into the mixture, at the expense of dry feed (w/w): 100, 200, or 300 g/kg of dry soil (diets 2 to 4) or 50, 100, or 150 g/kg of dry plants (diets 5 to 7). Diet 8 contained per kg 100 g of dry soil and 100 g of dry plants while 200 g of dry soil and 50 g of dry plants were incorporated into diet 9 (Table 1). Diets were presented as pellets of 2.5 mm in diameter.

2.2. Birds and management

The experiment was conducted under the guidelines of the French Ministry of Agriculture for Animal Research. One hundred and twenty, one-day-old and mixed sexed chicks of a commercial slow-growing line (JA657®, Hubbard SA, Quintin, France) were raised as a group with a starter feed up to the age of 28 days and with the previously mentioned commercial growth feed up to the age of 38 days. At this age, each chicken was weighed and the 90 birds closest to the mean weight of 753 g were kept in individual cages during the following 8-day experiment. Each bird was randomly assigned to one of the nine experimental diets and each diet was replicated ten times (one bird per cage). After a 5-day adaptation period, excreta from each bird were collected in their entirety during the last three days of the experiment, according to Bourdillon