

Research article

Chitin supplementation in the diets of captive giant anteaters (*Myrmecophaga tridactyla*) for improved gastrointestinal function

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Keywords:

Myrmecophaga tridactyla, chitin, diet, fibre

Article history:

Received: 8 September 2015

Accepted: 7 February 2017

Published online: 30 April 2017

Abstract

Clinical issues associated with keeping giant anteaters (*Myrmecophaga tridactyla*) in captivity may be linked with colon health and digestive function. Chronic loose stools are one such problem often suspected to be related to dietary intake. The objective of this study was to determine the acceptability, digestibility and faecal composition in a population of captive giant anteaters on four different experimental diets, including a baseline diet (B) comprising high fibre primate biscuit and dry feline diet, ground and mixed together then blended with water, or a commercial dry insectivore diet (INS), also ground and mixed with water. Other treatments included addition of 5 or 10% of dry matter (DM) as ground chitin added to B. No difference in faecal DM or faecal organic matter content was observed across all experimental diets; faecal ash was increased on B5 compared to B10 or INS treatments, indicating a possible impact on mineral nutrition. Similarly, no differences were observed in DM digestibility, or neutral detergent fibre (NDF) or acid detergent fibre (ADF) fermentation across all diets. The majority of the dietary components of the four different diets are highly digestible and/or fermentable (> 90% for all except fibre fractions). Crude fat and ADIN digestibility, and calcium and magnesium absorption were significantly higher in the diet that was formulated containing 5% chitin. Apparent digestion of NDF (82-91%) or ADF (74-88%), as measures of chitin, did not differ statistically among the diets in this study; ranges of disappearance of these components are high compared to other mammals, but within the ranges reported for other species.

Introduction

Giant anteaters (*Myrmecophaga tridactyla*) have specialised feeding needs and, in captivity, can present a unique nutrition challenge. In nature, giant anteaters consume mainly ants and termites, but the natural diet is not feasible in captivity (Stahl et al. 2012). Chronic loose stool is a nutrition-related clinical disorder associated with maintaining captive giant anteaters and has been reported in 27% of North American institutions in a recent health care survey (Morford and Meyers 2003). Faeces produced by captive giant anteaters are unlike that of the formed stools seen in free-ranging animals – often pasty to liquid in consistency – and quite prevalent in captivity such that poor stool quality has been accepted as typical or normal (Morford and Meyers 2003). Various dietary reformulations have been employed to alleviate chronic loose stools in managed anteaters, including providing more cellulose as a dietary ingredient to increase dietary fibre and improve faecal consistency (Morford and Meyers 2003). Cellulose, however, may not be the most suitable substitute fibre for insectivores. Dietary chitin from ant and termite exoskeletons consumed by free-ranging giant anteaters has been estimated at 5-17% of

dry matter (DM) (Redford and Dorea 1984), with only limited cellulose intake that might be present in insect gut contents.

Both physical and chemical differences between chitin and cellulosic material added to diets for managed insectivores may be important to consider, when supplements are used to mimic the insect-containing wild diet. While structurally similar to cellulose as a carbohydrate polymer, chitin differs from cellulose in that one hydroxyl group on each monomer is substituted with an acetyl amine group (Stevens 1995), making it a glycoprotein rather than polysaccharide. This configuration also allows for increased hydrogen bonding between adjacent polymers, which strengthens the chitin-polymer matrix. In nature (Beier 2013), chitin varies in the degree of deacetylation and can be deacetylated to chitosan or possibly even cellulose-like forms through deamination. Endogenous chitinase enzymes have been identified in various insectivorous species (Jeuniaux and Cornelius 1997). Thus chitin may serve not only as a potentially digestible dietary ingredient (for animals with appropriate adaptations), but physical features may also contribute by supporting normal microbial flora and potential fermentation, slowing transit time, and altering mineral and/or water absorption.

Objectives of this study were to determine the acceptability and utilisation of a baseline diet fed to captive giant anteaters, with or without added chitin at two inclusion levels or, alternatively, when fed a commercial diet developed specifically for insectivorous species. Digestive/colonic function was assessed through quantification of digestibility of dietary components and mineral absorption, with the working hypothesis that faecal fibre and water composition would improve (higher fibre, lower water content) with the addition of chitin, and/or an insectivore-specific commercial feed.

Methods

All methodological procedures were approved by Institutional Animal Care and Use Committees (IACUC) at both the University at Buffalo and Nashville Zoo at Grassmere, Tennessee. Animals were housed individually within a barn designed for giant anteaters in an AZA (Association of Zoos and Aquariums) accredited facility; indoor pens were connected to a respective enclosed outdoor pen. The individual housing allowed for the animals to be shifted to the outside or to adjacent indoor housing by sliding doors which are controlled by the zoo staff. Within the individual enclosures, anteaters were provided with a water tub for enrichment and bathing. Since anteaters commonly defecate in water tubs, during the faecal sample collection period the water tubs were emptied but left in the enclosures.

Diets were presented in non-tipping dog bowls that were placed on the floor of each enclosure, with anteaters fed twice daily at approximately 0800 and 1600 h. Diets were left within the enclosures up to 2 hours, based on rate of consumption and zoo keeper schedule. Within 3 hours following food presentation after each meal, individuals were shifted to an adjacent housing or outside area, and leftovers were collected from the feeding bowl or scraped off the floor with a putty knife. All orts were placed

into labelled bags, weighed, then frozen at -20°C. Whenever faecal samples were observed, individuals were shifted and the faecal samples were collected from the floor or dry tub with a putty knife, weighed and stored frozen at -20°C.

Diet Treatments and Analyses

Six adult captive giant anteaters were trialed on four experimental diets including a baseline (B), baseline with or without added chitin (+5% chitin (B5) or + 10% chitin (B10)), and a commercial insectivore diet (INS). The baseline diet comprised 220 grams Mazuri R Exotic Feline – Large #5M53 (Mazuri, St. Louis, MO) and 220 grams Mazuri R Primate Browse Biscuit M#5MA4, (Mazuri, St. Louis, MO) daily per animal. Treatments including 5% or 10% chitin (+22 or +44 g, respectively) were added (practical grade chitin from crab shells, catalogue number C7170, Sigma Chemical, St. Louis, MO) to B. INS consisted of 440 grams Mazuri R Insectivore Diet #5MK8 (Mazuri, St. Louis, MO) per day. All dry ingredients were ground to a fine meal, mixed with approximately 3.25x water (wt/wt: 1430 ml (B) to 1580 ml (B10) and blended to the consistency of a slurry-like gruel before feeding. One smaller female was fed the same diet treatments as other individuals, but amounts were scaled to approximately 80% of total volume due to reduced appetite.

Each experimental diet was fed sequentially over a two-week period; the first week (days 1-7) served as a dietary adaptation period and the second week (days 8-14) served as the experimental week. The diets were fed in the order: B5, B10, B and INS. DM contents of diets, chitin, uneaten diet (orts) and faeces were calculated from fresh samples. All faeces and orts for each animal were individually pooled (by diet) and analysed for DM, organic matter (OM), neutral detergent fibre (NDF), hemicellulose (HC), acid detergent fibre (ADF), acid insoluble ash (AIA), starch, acid detergent insoluble nitrogen (ADIN), ash, crude protein (CP), crude fat (CF), calcium (Ca), magnesium (Mg), phosphorus (P),

Table 1: Nutrient composition of diets fed to adult giant anteaters (n=6). All nutrients except water presented on a dry matter (DM) or organic matter (OM) basis.

| Dietary Treatment | Baseline (B) | B5 (Baseline + 5% chitin) | B10 (Baseline + 10% chitin) | INS (Commercial Insectivore Diet) |
|--------------------|--------------|---------------------------|-----------------------------|-----------------------------------|
| <i>Nutrients %</i> | | | | |
| Water | 77.6 | 77.1 | 77.8 | 78.8 |
| NDF (OM) | 24.2 | 27.5 | 30.6 | 32.5 |
| ADF (OM) | 13.9 | 16.9 | 20.9 | 14.2 |
| Hemicellulose (OM) | 9.4 | 9.2 | 9.0 | 18.2 |
| Starch (DM) | 20.7 | 19.8 | 18.9 | 17.8 |
| ADIN (DM) | 0.12 | 0.41 | 0.67 | 0.14 |
| Ash (DM) | 7.8 | 7.5 | 7.3 | 6.9 |
| Crude Protein (DM) | 30.6 | 30.5 | 31.5 | 29.8 |
| Crude Fat (DM) | 9.3 | 8.5 | 6.7 | 10.9 |
| <i>Minerals</i> | | | | |
| Sodium (DM) | 0.24 | 0.34 | 0.31 | 0.34 |
| Potassium (DM) | 0.78 | 0.74 | 0.72 | 0.75 |
| Phosphorus (DM) | 0.97 | 0.99 | 0.94 | 1.02 |
| Calcium (DM) | 1.67 | 1.74 | 1.67 | 1.40 |
| Magnesium (DM) | 0.14 | 0.13 | 0.12 | 0.15 |
| Na:K Ratio | 0.31 | 0.46 | 0.43 | 0.45 |

potassium (K) and sodium (Na). Acid insoluble ash (AIA) was used as an internal marker to calculate the apparent digestibility by determining AIA content in relation to end point analysed. Fibre analysis was determined sequentially as described by Van Soest et al. (VanSoest et al. 1991). ADIN, corrected for ash, was used as a surrogate marker for chitin (Jackson et al. 1992; Finke 2007).

Statistical analysis

Repeated measures ANOVA with post hoc analysis were used to determine differences between apparent digestibilities, apparent mineral absorption rates and the Na⁺ to K⁺ ratio, with Fisher's post hoc analysis to determine statistical significance with the main effect being dietary trial within animals. Regression analysis was used to determine true digestibility, mineral absorption and

the relationships between dietary components and faecal DM, and faecal mineral composition with faecal DM. All values are expressed on either a DM or OM basis with respective standard error of the mean (SEM). Statistical significance was considered at $P < 0.05$.

Results

Composition of the treatment diets fed is displayed in Table 1. Fibre fractions NDF (24-33%) and ADF (14-21%) ranged widely among the four diets on an organic dry matter basis (OM); HC was twice as high, and comprised >50% of NDF in INS as compared to all other diets (9 vs. 18%). B10 contained the highest ADF and ADIN concentrations, reflecting the higher addition of chitin. Mineral

Table 2: Dry matter consumed, dietary acceptance and faecal composition of 4 diets fed to giant anteaters.

| Dietary Treatment | Baseline (B) | B5 (Baseline + 5% chitin) | B10 (Baseline + 10% chitin) | INS (Commercial Insectivore Diet) |
|------------------------------------|----------------|---------------------------|-----------------------------|-----------------------------------|
| DM consumed, g/day | 308.0 ± 33.9 a | 334.0 ± 33.7 b | 330.0 ± 31.2 a,b | 348.0 ± 27.1 b |
| Dietary Acceptance, % | 77.5 ± 8.6 a,b | 81.5 ± 8.7 a,c | 75.7 ± 7.5 b | 85.5 ± 6.3 c |
| Starch consumed, g/day | 63.7 ± 7.0 | 66.0 ± 6.7 | 62.5 ± 5.9 | 61.9 ± 4.8 |
| NDF consumed, g/day | 71.7 ± 7.9 a | 89.2 ± 9.0 b | 98.4 ± 9.3 b | 113.0 ± 8.8 c |
| HC consumed, g/day | 30.2 ± 3.3 a | 32.0 ± 3.2 a | 30.9 ± 2.9 a | 65.5 ± 5.4 b |
| ADF consumed, g/day | 41.5 ± 4.6 a | 57.2 ± 5.8 b | 67.5 ± 6.4 c | 48.2 ± 3.8 d |
| ADIN consumed, g/day | 0.4 ± 0.04 a | 1.3 ± 0.1 b | 2.2 ± 0.2 c | 0.5 ± 0.1 a |
| Ash consumed, g/day | 23.9 ± 2.6 | 25.2 ± 2.5 | 24.3 ± 2.3 | 23.6 ± 1.8 |
| Protein consumed, g/day | 94.2 ± 10.4 | 101.9 ± 10.3 | 104 ± 9.8 | 103.7 ± 8.1 |
| Fat consumed, g/day | 28.6 ± 3.2 | 28.4 ± 2.9 | 22.1 ± 2.1 | 37.9 ± 3.0 |
| <i>Faecal Characteristics</i> | | | | |
| Faecal Dry Matter, % | 22.8 ± 1.7 | 24.3 ± 1.8 | 21.5 ± 1.2 | 23.2 ± 2.1 |
| Faecal OM, % | 16.4 ± 2.7 | 16.5 ± 0.5 | 16.6 ± 0.8 | 18.3 ± 1.6 |
| Faecal Ash, % | 6.4 ± 0.8 a,b | 7.8 ± 1.7 b | 4.8 ± 0.3 a | 4.9 ± 0.5 a |
| Faecal OM to Ash Ratio | 2.7 ± 0.2 a,b | 2.5 ± 0.6 a | 3.4 ± 0.1 b | 3.8 ± 0.2 b |
| <i>Apparent Digestibility</i> | | | | |
| DM, % | 90.3 ± 2.4 | 95.1 ± 2.0 | 88.6 ± 1.5 | 90.2 ± 2.0 |
| Starch (DM), % | 99.0 ± 0.3 | 99.5 ± 0.3 | 98.6 ± 0.2 | 98.9 ± 0.2 |
| NDF (DM), % | 81.7 ± 4.6 | 90.6 ± 4.2 | 82.9 ± 2.1 | 85.1 ± 3.0 |
| Hemicellulose (DM), % | 87.2 ± 3.7 | 94.4 ± 2.3 | 88.0 ± 2.0 | 93.0 ± 1.4 |
| ADF (DM), % | 77.5 ± 5.4 | 88.4 ± 5.3 | 80.5 ± 2.4 | 74.2 ± 5.2 |
| ADIN (DM), % | 75.1 ± 6.4 a | 91.9 ± 5.4 b | 89.8 ± 1.1 b | 74.3 ± 6.2 a |
| Crude Protein (DM), % | 92.4 ± 2.0 | 96.6 ± 1.6 | 90.8 ± 1.3 | 93.0 ± 1.5 |
| Crude Fat (DM), % | 96.3 ± 0.9 a | 98.9 ± 0.4 b | 96.0 ± 0.6 a | 96.2 ± 0.8 a |
| <i>Apparent Mineral Absorption</i> | | | | |
| Sodium, % | 85.6 ± 9.4 | 97.8 ± 0.9 | 93.9 ± 1.6 | 92.5 ± 3.7 |
| Potassium, % | 91.0 ± 2.2 | 96.1 ± 1.7 | 89.4 ± 1.3 | 90.0 ± 2.1 |
| Phosphorus, % | 74.4 ± 6.4 | 89.5 ± 4.6 | 72.9 ± 5.9 | 79.8 ± 4.6 |
| Calcium, % | 67.4 ± 7.7 a | 87.6 ± 5.1 b | 62.4 ± 5.8 a | 69.8 ± 6.4 a |
| Magnesium, % | 68.9 ± 7.2 a | 87.4 ± 5.2 b | 61.2 ± 5.7 a | 72.1 ± 5.5 a |
| Na:K Ratio | 0.36 ± 0.17 | 0.26 ± 0.06 | 0.26 ± 0.08 | 0.29 ± 0.11 |

a,b,c,d Values with a common letter are not significantly different based on Fisher exact, $P < 0.05$.

Values are the average of 6 animals on each of the four diets ± SEM except for two animals on the B5 diet due to collection errors and animal illness.

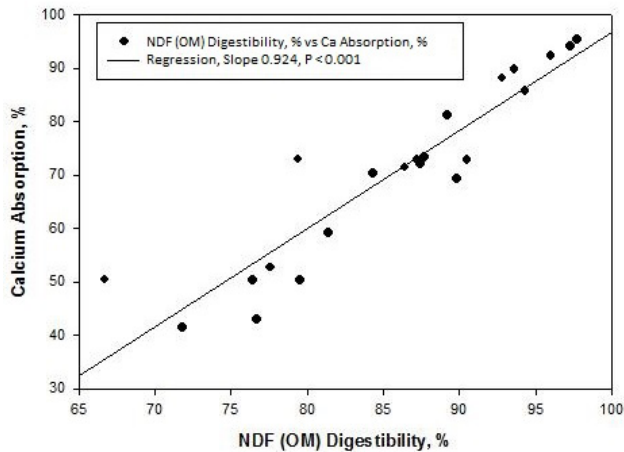


Figure 1: NDF fermentability was positively related to calcium absorption.

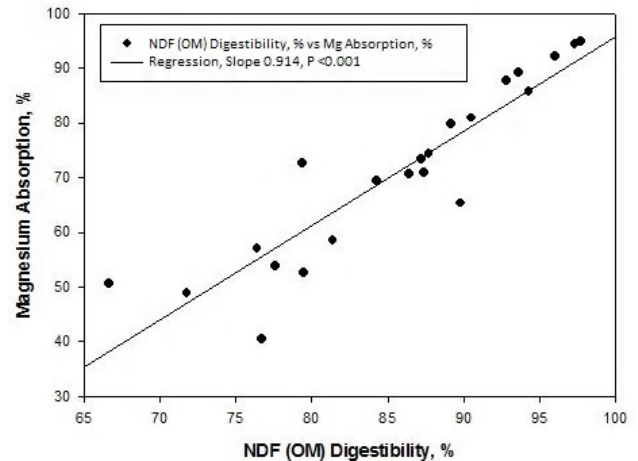


Figure 2: NDF fermentability was positively related to magnesium absorption.

content was similar across all four diets. Data from two anteaters on the B5 diet were excluded from all analysis due to illness (unrelated to the study) and collection issues. Intake of various nutrients varied among treatments (Table 2), except for starch and ash. Treatment diets B5 and INS appeared more palatable compared to B and B10 diets, both in terms of total consumption and percent consumed. The amount of total NDF consumed was highest for animals consuming the INS diet, due to its HC content.

However, the diet treatments with added chitin (B5 and B10) contributed significantly more insoluble dietary fibre (ADF and ADIN) than either the B or INS diets.

Among the diets, there was no difference in faecal DM or OM or apparent digestibility of DM (90.7%), starch (98.9%), CP (92.9%), HC (90.3%), NDF (OM) (85.6%), or ADF (OM) (81.3%). Percent DM digestibility was not related to dietary NDF (OM), ADF (OM) or HC (OM), starch, ADIN or chitin corrected for ADIN consumed. A negative relationship was found between NDF (OM) intake and faecal ash content ($P=0.037$). Faecal ash content was significantly greater in animals consuming B10 and INS diets compared with the B5 diet. Faecal OM/ash was significantly higher on the B10 and INS diets, with the INS diet 50% higher than B5.

True digestibility of DM, determined using Lucas plots (Lucas 1960), averaged 88% across all diets. True digestibility of ADIN across all diets was 89%, and was statistically higher for both chitin-added diets. Adding chitin at the 5% and 10% levels increased ADIN fermentation by 16.8% and 14.7%, respectively.

The INS diet was also found not to have a significant slope for the ADIN or ADF (OM) true digestibility endpoints. Apparent and true digestibility of starch (99%) was the highest compared to other nutrients in all diets. Calcium and Mg absorption were significantly higher on the B5 diet compared to all other diets ($P < 0.05$). A significant relationship between the faecal Na^+/K^+ ratio was found between faecal water ($P < 0.001$) and dry OM ($P < 0.001$). Positive significant relationships were found between organic matter NDF digestibility and the absorption of Ca ($P < 0.001$; Figure 1) and Mg ($P < 0.001$; Figure 2).

Discussion

The digestion trials showed that both acceptability and digestibility of the B5 diet was not different from the commercial INS diet, suggesting that both might be viable alternatives to feeding the B diet as a higher fibre diet. One explanation for the increased B5

and INS diet acceptability (based on intake of an ad lib offering) could be the nutrient density, as these diets contained more added fibre, and dilution of overall nutrient content could be driving the increased intake, and subsequently the acceptance.

Maintenance energy requirements of giant anteaters (46-64 kg) have been determined at $347 \text{ KJ kg}^{0.75}/\text{day}$ (Stahl et al. 2012). Animals in this study averaged 45 kg, thus required about 6000 kJ (1400 Kcal) per day. Total calculated energy of daily diets consumed in this study ranged from ~ 4100 to ~ 4800 KJ, or approximately 70 to 80% of expected energy needs, and animals maintained health and weights. Highly digestible components (starch, fat, and protein) in the baseline diet comprised about 62% of calculated energy needs, compared to about 70% of energy needs from the INS diet (due to its higher fat content), with the remainder presumably supplied by the NDF fibre and/or an unquantified soluble carbohydrate fraction (Table 1). The unknown 2-7% of DM missing from the total diet composition may be laboratory artifact or soluble organic compounds not analyzed. NDF appears to be contributing only about 10-12% of dietary energy from DM digestion calculations.

The faecal DM percentage was not significantly different between the diet treatments; however, anteaters that ate the B5 chitin or INS diets had higher percentages of faecal DM. Faecal DM ranged from 22% to 24%, and stool consistency ranged from liquid to soft and slightly formed, respectively (personal observation).

In birds, chitin digestibility has been shown to range from 7% (northern bobwhite) to 85% (king penguin); mice fed 16% chitin digested 19 to 59% (Jeuniaux and Cornelius 1997). Other studies with mammalian species have demonstrated chitin digestibility from 2% (musk shrew) to 39% (house mouse) (Akaki and Duke 1999). Apparent fermentation of NDF or ADF, as measures of chitin, did not differ statistically among the diets in this study; however, ranges of disappearance of these fractions (82 – 91% and 74 – 88% for NDF and ADF, respectively) are high for mammals, but within ranges reported for other species. Thus anteaters certainly appear to have the ability to utilize dietary fibre concentrations ranging from 24-33% NDF (OM basis) and 14-21% ADF (OM basis). Possible fermentation of dietary fibre may be related to the low metabolic body rate and recently demonstrated cecal fermentation potential (Carvalho et al. 2014) as well as large body size of giant anteaters; both can be linked with longer retention time and hindgut fermentation potential (Gull et al. 2014). Apparent fibre digestibility may also be related to the presence of microbial and/or endogenous chitinase resulting in more effective digestion of

chitin-containing diets (B5 and B10 diets) compared to the higher-proportion cellulose containing diets (B and INS). Hedgehogs, a mammal with suspected endogenous chitinase activity, have been demonstrated to more effectively digest chitin-containing diets compared to cellulose-containing diets (Graffam et al. 1998). It is possible that ADIN may be a useful marker for potential chitinase activity, but warrants further investigation.

There was a positive (nonsignificant) trend between ingested NDF (OM) and faecal DM ($P < 0.07$) and a significant positive relationship with the ratio of faecal OM to ash. When looking at the relationships among NDF (OM) digestibilities and other faecal fractions, NDF digestibility was highly correlated with faecal DM ($P = 0.002$, data not shown) and furthermore this NDF (OM) digestibility was strongly positively related to OM:ash ratio ($P = 0.001$, data not shown). This suggests that the increase in NDF (OM) digestibility is greater as fermentation increases, possibly due to improved microbial population activity. The findings of this study suggest that undigested dietary fibre may result in wetter faecal material, with diarrhea or very loose stools in anteaters resulting possibly from too much indigestible dietary fibre.

Adding chitin at the 5% level increased Ca and Mg absorption by approximately 20%, although at the 10% chitin level, Ca and Mg absorption decreased by approximately 5% to 8%, or 8% to 11%, compared to the B and INS diets, respectively. The significant increase in Ca and Mg absorption with the 5% chitin addition supports the hypothesis that adding a potentially digestible fibre source, chitin, affected mineral absorption. This relationship is also supported by the strong positive correlation found between NDF (OM) and Ca and Mg absorption such that as NDF digestibility increases, Ca and Mg absorption increases. The significant negative correlation between faecal Na:K ratio and faecal water suggests that less Na is absorbed and therefore less water is absorbed.

Based on the results of this study, both the B5 chitin and INS diets appeared to be palatable and tended to be more digestible compared to the baseline diet or B10. INS was the best diet in terms of acceptance and resulted in higher faecal fibre content, but fibre digestibility did not differ statistically from other treatments. The B10 chitin diet had a high OM to ash ratio, resulted in visually, but not statistically, wetter faeces, and had the lowest acceptability of treatments. While the additional chitin ADF resulted in an increase in potentially fermentable fibre in anteater diets, both the 10% chitin and INS treatments may have negatively impacted Ca and Mg mineral nutrition.

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