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Oxalate and Calcium Flows at the Terminal Ileum of Pigs Following the Consumption of Test Diets Containing Fresh or Ensiled Taro Petioles

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Abstract

Taro (*Alocasia odora* L.) petioles are widely used as a component of pig diets in Central Viet Nam because they contain a range of important nutrients such as protein and carbohydrates. However, the petioles are known to contain high levels of oxalates which can substantially reduce the absorption of calcium from the digestive tract. Six young crossbred (Large White x Mong Cai) pigs (mean weight 56 ± 1.6 kg were fitted with PVC T-piece cannulas at the terminal ileum and then housed in individual metabolism cages. The pigs were fed either 50% fresh or 50% ensiled taro petioles along with rice bran and rice wine by-products. The experiment was designed to determine the flow of oxalates and calcium past the terminal ileum over a 24-hour period. Ensiling the taro petioles significantly reduced the total intake of oxalates in the test diets resulting in significantly ($p<0.05$) lower flows of total and soluble oxalate past the terminal ileum. Ensiling the taro petioles led to a significant reduction in the % soluble oxalates absorbed prior to the ileum compared to the fresh diet (30.6 vs 34.5%). The total calcium intake of the pigs fed the two diets were similar but the flow of calcium past the ileum was significantly reduced in the pigs fed the ensiled petiole diet. The total amount of calcium bound to insoluble oxalate passing the terminal ileum were similar for the fresh or ensiled diets (23.8 vs 28.0%) when compared to the total intake of calcium.

Keywords: Total, Soluble, Insoluble Oxalate, Calcium, Pigs, Silage, Taro

Introduction

Taro (*Alocasia odora L.*) has been shown to yield up to 370 t/ha/year of fresh foliage (leaves and foliage) in the hot and humid conditions found in Central Viet Nam [1]. The plant grows rapidly and efficiently sequesters carbon from the atmosphere at a greater rate than tropical forests [2]. Taro leaves and petioles are rich in protein and soluble carbohydrates [3-5] and there is positive economic advantage to use locally grown forage for feeding to pigs in this region. The main limitations, however, is the antinutritive effect of high levels of oxalates, which can form nonabsorbable salts with Ca, Fe and Mg ions, rendering these minerals unavailable [6]. Holloway et al. [7] were the first to report the total oxalate of nine taro cultivars, ranging from 278 to 574 mg/100 g wet matter (WM), with the edible leaves generally having lower levels of total oxalates than the leaves considered as being inedible. Hang et al. [8] showed that the total oxalate content of taro leaves ranged from 4.43 to 4.68 g/100 g DM, while the soluble oxalate content ranged from 0.81 to 1.76 g/100 g DM. These studies suggested that up to 35% of the total calcium in taro forage was locked up as insoluble calcium bound to oxalate. Furthermore, intake of the soluble oxalate fraction in taro forage could further decrease the absorption of several important essential minerals, such as iron and magnesium in addition to calcium [9,10].

Taro forage can be fed to pigs up to 50% of the diet, supplemented with rice bran and rice wine by-products both locally sourced materials [11]. The taro forage was fed either fresh or ensiled. The preparation taro forage silage allows the herbage to be stored for future use but lactic fermentation during storage may lead to an improvement in the nutrient availability of the final product [11]. There is also a possibility that lactic acid fermentation in the silage might lead to a reduction in the soluble oxalate content of the herbage. This possibility was investigated by Wadamori et al. [12] who investigated lactic acid fermentation during the preparation of kimchi. They showed that a 23% reduction in soluble oxalate could be achieved after five days of fermentation. Wang [13] reported that the high oxalate concentrations in forage could be reduced by fermentations that occur during the ensiling process. Fermentation with molasses resulted in a 50% reduction in the total oxalates [13-15] showed that the biological value of taro leaves could be increased, from 65 to 77%, by cooking or ensiling methods. Making silage from taro petioles may well offer the same positive effects by reducing the soluble oxalate content and improving the digestibility of other important nutrients such as protein.

The requirement for calcium in the diet of pigs growing from 40-60 kg body weight is 0.7% [16], this must be supplied by the raw or ensiled taro petioles. Most calcium absorption occurs in the small intestine [17-19] and is affected by the type of diet fed [17]. So, to investigate the changes in absorption of soluble oxalate and calcium in the small intestine it is necessary to sample the digesta that passes the terminal ileum. Analysis of faecal samples may lead to inaccurate observations as hind gut microbes may well carry out further fermentations that will be of no benefit to the pig's metabolism. Therefore, this study involved an investigation of the overall effects of silage fermentation on the levels of calcium and oxalates passing the terminal ileum over 24 h of young growing pigs.

Materials and Methods

Harvesting and processing of taro petioles/leaves

Approximately 100 kg of Mon Ao Trang (*Alocasia odora L.*) (50

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kg leaves, 50 kg petioles) were harvested from two farms with similar sandy soils located close to the university experimental farm in central Viet Nam. The plants were not fertilised during the growing season but were irrigated to maintain steady growth throughout the experimental period. Leaves and petioles were chopped separately into small pieces (10 - 20 mm) using an electric forage cutter, they were spread out in the shade and wilted for 24 h to reduce the moisture content to 65% when wilted. During wilting the forage were turned and ventilated to prevent moulds from developing and mixed with 4% sugar cane molasses (by fresh weigh). The mixed forage was then packed into nylon bags (500 mm x 700 mm x 200 µm), excess air was removed, and the bags were heat-sealed. The silage was allowed to ferment for 14 d at 36 ± 5°C. The ensiled leaves or petioles formed the basis of the diet. For the fresh taro treatment, taro was harvested every day at 5 AM.

Three representative samples (~300 g) of chopped leaves or petioles and silage were sampled, dried at 65°C for 18 h and then ground to a fine powder using a Sunbeam Multi-Grinder (Model no. EMO 400, Sunbeam Corporation Limited, NSW, Australia) before being sealed in plastic bags until analysis commenced. The residual moisture of each sample was determined in triplicate by drying for 24 h in an oven to a constant weight at 105°C [20].

Animals and sampling

Six castrated Large White male pigs (48 ± 2.8 kg) were fitted with simple T-cannulas [21] and allowed to recover on a standard pig growing diet forthree weeks. All pigs were vaccinated against hog cholera and pasteurellosis, and de-wormed two weeks before start of the experiment. Before commencing the experiments, animal housing and experimental procedures were reviewed and approved by the Hue University Committee on Animal Care in accordance with the Animal Care Policies and Guidelines Involving the Use of Animals. The pigs were surgically fitted with post-valve T-caecum cannula [22,23] for the collection of ileal digesta andthen housed individually in metabolism cages two weeks prior to being introduced to the experimental rations. Three pigs were assigned to each of the following two treatments fresh or ensiled taro petioles.

Collection of ileal digesta

Restricted amounts of each diet were offered to the pigs at a level of 80% of observed voluntary intake during the one-week adaptation period. Pigs fitted with PVTC-cannulas were individually housed in metabolismcages and chromium oxide was used as a digesta flow marker [21].

The pigs were fed the experimental diets at 6 am on each collection day. After 60 min small amounts of uneaten feed was collected, weighed and samples taken for analysis. Water was available at all times from a nipple drinker. The total flow of ileal digesta was collected from the T piece canular of each pig. The collection periods were from 6-10, 14-18, and 22-24 hours of first day and 10-14, 18-22 hours of the second day collection. The total digesta was collected into a cooled container (4^0C). At the end of each 4-hour collection period the digesta was weighed, mixedand a 300 g returned to the ileum of each pig before the next collection period commenced.

Oxalate and calcium analysis

The total oxalate contents of the individual finely ground samples (~0.5 g) of feed and digesta samples were determined in duplicate, using the method outlined by Savage et al. [24]. The final oxalate values of all samples were converted to mg/100 g DM of the original material.

The calcium content of each sample of the ileal digesta was determined by atomic absorption spectrometry AOAC method 935.13 [25]. The proximate, oxalate and calcium content of the individual components (% DM) of the fresh and ensiled diets are shown in Result and the proximate, oxalate and calcium content (% DM) of the diets fed to the pigs are shown in Result 2. The DM, CP of samples were determined by standard methods of AOAC [25]. The Cr_2O_3 in feed, ileal digesta was determined by atomic absorption spectrometry after ashing [26].

Statistical analysis

Data were analyzed by ANOVA using a General Linear Model (GLM) in Minitab version 16 (Minitab Ltd., Brandon Court, Progress Way, Coventry, UK). Tukey's pair-wise comparison was used to determine differences between treatments at $P<0.05$.

Results

The composition of the ingredients of the two taro-based diets showed that a balanced diet for pigs could be constructed using 50% fresh or ensiled taro petioles (in DM) supplemented with rice bran and rice wine by-products. The overall mix contained adequate amounts of protein and calcium, but a feature of the overall diet was the high content of total and soluble oxalates which were found in the taro petioles. The ensiled taro petioles contained 50.9% less total oxalates compared to the fresh taro petioles with the soluble oxalates showing a 67.4% reduction following ensilage of the petioles. Rice bran contained smaller amounts of oxalates compared to the rice wine by-product. Although rice bran contained small amounts of total oxalate it contained relatively high levels of insoluble oxalates (60% of the total). Result shows the composition of the two diets which were constructed to contain 50% fresh petioles or 50% ensiled taro petioles and calcium, oxalate composition (% DM) of the experimental diets.

Result confirms that the total and soluble oxalate intake of the pigs fed ensiled taro petioles were considerably reduced when compared to the intake of a test diet containing an equivalent amount of fresh taro petioles. This resulted in a significantly ($p<0.05$) reduced flow of total and soluble oxalate past the terminal ileum over the following 24-hour period. The data shows that the absorption total and soluble oxalates prior to the terminal ileum were significantly reduced ($p<0.05$) when the flows were compared between the fresh vs the ensiled taro diets (61.2 and 58.5% respectively). Overall, when allowance for the reduction in the total and soluble oxalate contents of the original test diets was made the total oxalate absorbed as a % of the total intake was reduced for the ensiled diet. However, the % soluble oxalate absorbed prior to the terminal ileum were the same for the two test diets.

Result shows that the total calcium intake and the total calcium flowing past the terminal ileum (g/24h) were comparable between the two test diets. The calcium content in insoluble oxalate was calculated assuming that insoluble oxalate was solely made up of calcium bond to the oxalate molecule and was, therefore, calcium oxalate which contains 31.3% calcium. The amount of calcium bound to insoluble oxalate passing the terminal ileum was 89.6% lower ($p<0.05$) in the pigs fed the ensiled taro petioles however, the insoluble calcium expressed as a % of the total calcium intake of the pigs was significantly lower ($p<0.05$) as the fresh taro diet contained higher amount of total calcium. This data suggests that the pigs would absorb sufficient calcium from both taro petiole diets to maintain healthy bone growth and the diets would meet the NRC [16] requirements for calcium in the diet of growing pigs.

Discussion

High levels of oxalates in foods can have several effects in the digestive tract and if absorbed, on the animal's metabolism. Fortunately, only between 6 to 14% of ingested oxalates are absorbed into the body from the intestinal tract [27,28]. Absorption appears to occur all along the intestinal tract with approximately half occurring in the small intestine and half in the large intestine [27]. The bioavailability of oxalate in feeds with a high levels of soluble oxalate content was much lower than that of sodium oxalate. The amount of soluble oxalate is usually assessed as the amount of oxalate in feed that is soluble after homogenization of the feed in hot water [24]. The insoluble oxalate is assumed to be crystalline calcium oxalate. Curhan et al. [29] reported an inverse relationship between calcium intake and stone formation in humans. It was suggested that this effect was due to calcium binding to soluble oxalate in the intestinal tract thus limiting its uptake. A similar effect of calcium on oxalate excretion was observed in dietary studies [29]. However, the presence of oxalate-degrading organisms in the intestinal tract, particularly *Oxalobacter formigenes*, may lower the amount of oxalate available for absorption. The absence of *Oxalobacter* colonization in the digestive tract in humans with increased stone formation and increased oxalate excretion in stone formers [30-32] as higher levels of soluble oxalate would be available for absorption in the small intestine. This effect may well occur in other simple stomached animals, but no studies have been carried out. The development of kidney stones has not been observed in pigs fed taro leaves or petioles for their entire productive life.

Hatch and Freel [33] showed that soluble oxalates were actively absorbed in the small intestine with limited passive absorption occurring in the ileum. The presence of soluble oxalates in the small intestine, particularly for the taro leaf diets had a significant effect on the digestibility of calcium in this region [33,34], presumably the soluble oxalates chelated with calcium in the small intestine preventing its absorption. The higher digestibility coefficients for calcium and oxalate seen in pigs fed ensiled taro leaves compared to fresh leaves, may be the effect of ensiling reducing the pH from 7.1 to 3.6in the silage as the lactic acid content increased after 14 days (from 0.78 to 1.52% of ensiling, and this would facilitate the digestibility and absorption of Ca and Mg [8]. It has been shown that calcium absorption in rats was decreased by soluble oxalate, and it has also been reported that calcium absorption in adults after eating vegetables rich in oxalate was significantly lower when compared to vegetables with a low oxalate contents [35,36]. The higher levels of oxalate intake in pigs fed taro leaves or petioles the central district of Viet Nam does not appear to have significant effects on the calcium metabolism and no reports of weakened bone structure have been reported. This study has clearly shown that ensilage of taro petioles significantly reduced the total and soluble oxalate content of the ensiled leaves which reduced the amount of oxalates absorbed prior to the terminal ileum. Overall, the inclusion of fresh or ensiled taro resulted in balanced diet for growing pigs . This study therefore confirms that the inclusion of up to 50% dry matter of petioles or ensiled petioles is a safe nutritious inclusion of a locally produced fodder crop in the diet of growing pigs.

Conclusions

Raw and ensiled taro petioles are useful components in growing pig diets as they provide locally and cheaply grown protein and carbohydrates. The petioles contain moderate levels of soluble oxalates which are reduced to some extent during the ensiling process. Raw and ensiled taro petioles contain adequate levels of calcium for a growing pig diet however, a mean of 26% of the total calcium intake is bound to oxalates at the terminal ileum.

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