THE EFFECT OF OXALATE ON THE AVAILABILITY OF MINERALS IN PIG DIETS CONTAINING FRESH OR ENSILED TARO LEAVES

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Abstract

Taro (*Alocasia odora*) leaves are widely used as a component of pig diets in Viet Nam because they provide useful amounts of protein and carbohydrates in the diet. However, the leaves are known to contain high levels of oxalate, and these can substantially reduce the of absorption of Ca and Mg 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 caecum cannulas and then housed in individual metabolism cages. The pigs were fed a control diet consisting of rice bran, maize, rice wine by-product and fish meal in a double 3 x 3 Latin square design in which the other two diets were replaced with 50% fresh or 50% ensiled taro leaves (both on DM basis). The experiment was designed to determine the effect of feeding fresh taro leaves and taro leaf silage on the retention of N, Ca and Mg as both of these feeds contain oxalates, which may reduce the absorption of some nutrients from the leaves.

The ileum and total tract digestibility coefficients of the crude protein, Mg and Ca were lower when the diets containing fresh or ensiled taro leaves were fed compared to the control diet. The retention of nitrogen, Ca and Mg was also lower in the taro leaf diets than in the control diet. However, the diet containing ensiled taro leaves showed improved retention of Ca and Mg compared to the fresh leaf diet.

Keywords: calcium, digestibility, magnesium, retention

Introduction

Taro (*Alocasia odora*) was reported to yield up to 370 t/ha/year of fresh foliage (leaves and petioles) with the leaves representing some 50% of the foliage dry matter (DM) (Toan and Preston 2010). This implies a rate of sequestration of carbon from the atmosphere of 35 t/ha annually which can be compared with the average tropical forest estimated to sequester about 8 t/ha/year (Myers and Goreau 1991). Taro leaves are rich in protein (21to 26% of DM) while the petioles are rich in soluble carbohydrates (Hang and Preston 2009; Rodríguez and Preston 2009; Hang and Kien 2012). There is thus a strong incentive to develop the use of taro foliage in diets for pigs in tropical regions as a partial response to the emergency posed to the earth's climate by global warming.

The main limitation of taro foliage as a feed for pigs is the presence of oxalates, which can form nonabsorbable salts with Ca, Fe and Mg ions, rendering these minerals unavailable (Savage et al 2009). Holloway et al (1989) were the first to report the total oxalate of nine 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 (2016) 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. Furthermore, intake of the soluble oxalate fraction in taro leaves will further decrease the absorption of several important, essential minerals, especially calcium (Liebman and Chai 1997; Liebman and Costa 2000).

Most calcium absorption occurs in the small intestine (Partridge 1978; Liu et al 2000; Bohlke et al 2005) and is affected by the type of diet fed (Partridge 1978). To avoid manipulation by microbes in the hind gut, Cunningham et al (1962) developed and described a cannula that could be surgically inserted into the distal ileum of growing pigs. Then, later, a post-valve-T-cecum cannulation procedure was described by van Leeuwen et al. (1991).

The possibility that lactic acid fermentation of the herbage might lead to a reduction in the soluble oxalate content of silver beet fermented to make kimchi was investigated by Wadamori et al (2014). They showed that a 23% reduction in soluble oxalate could be achieved after five days of fermentation. Wang (1983) reported that the high oxalate concentrations in feed could be reduced by fermentations that occur during the ensiling process. Fermentation with molasses resulted in a 50% reduction in the total oxalates (Hang and Preston 2010; Tiep et al 2006). Moreover, it was found that the biological value of taro leaves could be increased, from 65 to 77%, by cooking or ensiling methods (Hang and Preston 2009). Making silage from taro leaves thus offers the possibility of reducing the soluble oxalate content.

Materials and methods

Harvesting of taro leaves

Approximately 50 kg of Mon Quang (*Alocasia odora*) leaves were harvested each day from two farms with similar sandy soils located close to 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. Mixed leaves (15 kg) were chopped into small pieces (10 - 20 mm) using an electric forage cutter and fed fresh (FTL diet) to the pigs. The remaining 35 kg of taro leaves were spread out in the shade and wilted for 24 h to reduce the moisture content from 85% when fresh to 62% when wilted. During wilting the leaves were turned and ventilated to prevent mould from developing. The wilted leaves were then chopped into small pieces (10 - 20 mm) and mixed with 4% sugar cane molasses (by fresh weight). The chopped wilted leaves were then packed into polethylene bags (500 mm x 700 mm x 200 μ m), excess air was removed and the bags heat-sealed. The silage was allowed to ferment for 14 d at 36 ± 5°C. The ensiled leaves formed the basis of the ETL diet

Three representative samples (~300 g) of chopped leaves and silage were 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. The residual moisture of each sample was determined in triplicate (Hortwitz and Latimer 2007) by drying for 24 h in an oven to a constant weight at 105°C.

Diets and feeding

Three dietary treatments were investigated: (i) the basal diet (control) consisting of maize, rice bran, rice wine by-product and fish meal (FM); (ii) the basal diet with the replacement of 50% of the DM by fresh taro leaves (FTL); and (iii) replacement of 50% DM by ensiled taro leaves (ETL). The composition of the main ingredients (g/kg DM) is shown in Table 1, while the ingredient composition (g/kg DM) and chemical analyses of the three experimental diets are shown in Table 2.

Table 1. Chemical composition of the main ingredients of the experimental diets (g/kg DM except dry matter

which is on fresh basis)

	ish Rice wine	Fresh taro	Ensiled taro
bran ^{Maize} m	eal by-product	leaves	leaves

Dry matter	895	867	895	100	200	143		
Crude protein	149	80.1	462	298	210	212		
Crude fiber	596	2.6	3.2	5.6	13.7	17.4		
Crude fat	170	51	82	-	42	33		
Ash	87	14	146	52	84	83		
Oxalate, mg/100g DM								
Total	430	300	300	200	7800	1930		
Soluble	180	140	130	80	3100	760		
Insoluble	250	160	170	120	4700	1170		

Table 2. Ingredient composition and chemical analysis of the experimental diets, the control diet

 (FM) or fed 50% of FM diet replaced by fresh taro leaves (FTL) or ensiled taro leaves (ETL)

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Ingredients (% of DM)	FM FTL	ETL					
Rice bran	39.0618.17	18.32					
Maize	39.0023.43	23.43					
Fish meal (FM)	16.00 2.00	2.00					
Rice wine by-product	5.00 5.00	5.00					
Fresh taro leaves (FTL)	50.00						
Ensiled taro leaves (ETL)		50.00					
Monocalcium phosphate	0.27	0.27					
MgO	0.18 0.19	0.16					
Cr_2O_3	0.50 0.50	0.50					
Lysine	0.10	0.10					
Meth+Cys	0.25 0.20	0.20					
Chemical analysis (% of DM)							
DM	90.0 48.8	49.5					
СР	17.0 17.0	17.0					
CF	4.1 8.2	7.6					
Lysine	0.8 0.8	0.8					
Meth+Cys	0.6 0.6	0.6					
ME (kcal/kg diet)	3100 3080	3060					
Ca	0.8 0.8	0.8					
Mg	0.37 0.37	0.37					
Oxalate, mg/100g DM							
Total	34.0 980.0	410.0					
Soluble	15.0 390.0	160.0					
Insoluble	19.0 590.0	240.0					

Restricted amounts of each diet were offered to the pigs at a level of 80% of observed voluntary intake during the adaptation period. Feeding times were 8:00, 10:00 and 15:00 h for the taro leaves and 6:00, 12:00 and 17:00 h for the other components of the diets. After 40 min small amounts of uneaten feed were collected, weighed and samples taken for analysis. Water was available at all times from a nipple drinker.

Animals and sampling

Six castrated male pigs (F1, Large White × Mong Cai) with an initial body weight of 56 ± 1.6 kg, and aged four months, were randomly allotted to the experimental diets. The experimental design was a repeated Latin square. The overall experimental period for each pig was 12 d; seven days for adaptation and five days for collection of feces and urine.

The pigs were vaccinated against hog cholera and pasteurellosis, and de-wormed two weeks before starting 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 a post-valve T-caecum cannula (Van Leeuwen et al 1991) for collection of ileal digesta and then individually housed in metabolism cages. A screen floor for faecal collection and a tray for urine collection was placed under each cage to allow for quantitative collections of urine and feces from each animal. Urine was collected into plastic bottles containing 10 mL of 20% sulphuric acid to prevent loss of ammonia. Urine was collected daily and weighed, with a 20% subsample stored at -20° C. Fecal samples were also stored at -20° C. All samples were mixed within pigs at the conclusion of the experimental feeding period. For the ileal digestibility determination, a total of nine digesta samples from each pig were taken during the last two days of collection. On each of these days, samples were taken every two hours between the morning and afternoon feedings, kept on ice and then frozen at -20° C. The digestibility of the components of each diet mix was calculated according to the chromium oxide marker in the feed (5 g/kg) (Sauer et al. 2000). The DM of feed offered and refused, and the DM and N, Ca and Mg in the feces and urine were determined according to standard AOAC methods (2007).

Analysis of diets, digesta and feces

The crude protein of the diets, and ileal and fecal samples were determined using standard AOAC methods (2007). The Cr_2O_3 in the feed, feces and ileal digesta was determined by atomic absorption spectrometry following ashing in a furnace at 550°C (Fenton and Fenton 1979). Mg and Ca were determined by atomic absorption spectrophotometry (AOAC 2007) (methods 968.08 and 935.13). The total oxalate contents of the individual finely ground samples (~0.5 g) were determined in duplicate, using the method outlined by Savage et al (2000). The final oxalate values of all samples were converted to mg/100 g DM of the original material.

Calculations

The retention of Ca and Mg within the body of each pig was calculated using the Petersen and Stein (2006) method, while the apparent total tract digestibility of Ca and Mg for each pig were calculated using the indicator technique published by Sauer et al (2000). The metabolizable energy (kj/kg) of each diet was calculated using the method published by NIAH (2001) where ME = (5.01 digestible protein + 8.93 digestible fat + 3.44 digestible fiber + 4.08 digestible nitrogen-free extractives) x 4.184.

Statistical analysis

Data were analysed by ANOVA using the 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 for both proximate and oxalate contents (Table 1) was similar to previously published values (Hang et al 2016b). The fresh taro leaves had very high total oxalate content but the levels were reduced by75% in the ensiled leaves; however, low levels of total and soluble oxalates were also observed in all the other ingredients. The three experimental diets were formulated to contain similar amounts of crude protein, metabolisable energy, calcium and magnesium (Table 2). Overall, the chemical analyses of the three experimental diets were very similar. However, the substitution of fresh taro or ensiled taro leaves to replace fish meal in the control diet led to a reduction in the rice bran and maize in the taro leaf diets to account for the high carbohydrate content of the taro leaves. The total oxalate content of the control fish meal diet was very low (34 mg/100 g DM). The addition of fresh taro leaves to the diet

led to a very large rise in the total oxalate content in the fresh leaf diet (980 mg/100 g DM) but this was reduced by 58% to 410 mg/100 g DM when the taro leaves were ensiled. The proportion of soluble oxalate in the fresh taro leaves diet was 39.8% of the total oxalate content. The same ratio of soluble oxalate to total oxalate content (39.0%) was also seen in the ensiled taro leaf diet. This confirmed that during silage fermentation both soluble and insoluble oxalates were degraded to the same extent.

There were differences in the coefficients of the apparent ileal digestibility of crude protein, Ca, Mg and oxalate among the treatments (Table 3). There were reductions in the ileal digestibility of crude protein, calcium and magnesium when the taro leaves and the taro silage diets were compared to the control diet. Similar reductions in the total digestive tract digestibility were observed for these parameters. However, ensiling the taro leaves did not affect the digestibility of the crude protein when compared to the fresh leaf diet, suggesting that taro leaves were highly digestible. The digestibility of the oxalate measured flowing past the ileum and at the rectum were very similar suggesting that oxalates were not absorbed in the large intestine. The oxalate content of the FTL diet was higher than in the control (FM) diet and in the ensiled taro leaf diet (FTL).

The higher levels of oxalate in the FTL and ETL diets may be responsible for the lower calcium digestibility coefficients observed at the ileum and rectum of the two taro-containing diets.

Table 3. Effect of ensiled taro leaves on apparent digestibility coefficients at the ileum and rectum, in pigs fed a rice bran, maize basal-diet and fish meal (Control) or with 50% of DM replaced by fresh taro leaves (FTL) or ensiled taro leaves (ETL)

Digestibility, %	FM FTLECL SEM	р
Ileum		
СР	0.68°0.65°0.62°0.0046	< 0.001
Ca	0.51°0.35 ^b 0.44 ^a 0.0048	< 0.001
Mg	0.31ª0.21 ^b 0.22 ^b 0.0011	< 0.001
Total oxalate	0.54°0.26 ^b 0.40 ^a 0.0041	< 0.001
Rectum		
СР	0.73 ^b 0.68 ^a 0.67 ^a 0.0051	< 0.001
Ca	0.56°0.38 ^b 0.49 ^a 0.0033	< 0.001
Mg	0.37ª0.27 ^b 0.29ª0.0012	< 0.001
Total oxalate	0.58°0.28 ^b 0.43 ^a 0.0041	< 0.001

The overall intakes of DM, nitrogen, calcium and magnesium did not differ among the experimental diets (Table 4). Nitrogen retention of the two taro diets were 27% lower when compared to the fish meal diet. The nitrogen digestibility values of the three diets were similar but the main difference occurred in the increased nitrogen output in the urine of the pigs fed the two taro leaf-based diets. The nitrogen retention of the pigs fed the ensiled taro leaf diet was higher when compared to the raw taro leaf-based diet. This suggests that the quality of the protein in the taro leaves was increased in the fermentation process. Hang and Preston (2009) reported earlier that the biological value of the protein in fresh taro leaves was increased from 63.2 to 73.8 in ensiled taro leaves.

Table 4. Mean values for balance of N, Ca and Mg in pigs fed the control diet (FM) or with 50% of the DM in the control diet replaced by fresh taro leaves (FTL) or ensiled taro leaves (ETL)

-	FM	FTL	ETL	SEM	p
DM intake, g/d	1458ª	1528ª	1436ª	29.91	0.090
N balance, g/d					
Intake	40.91 ^a	43.04 ^a	40.44^{a}	0.852	0.083
Feces	10.96	13.99	13.47	2.355	0.001
Digested	29.95 ^a	29.05ª	26.97ª	0.823	0.550
Urine	7.58°	13.47 ^a	9.83 ^b	0.608	0.001
N retention	22.37ª	15.57 ^b	17.13 ^b	0.555	0.001

N retention/N digested, %	75 ^a	54°	64 ^b	1.62	0.001
Calcium balance, g/d					
Intake	11.8 ^a	12.27ª	11.55ª	0.256	0.141
Feces	5.10°	7.65ª	6.00^{b}	0.177	0.001
Urine	1.67 ^b	2.33ª	1.88 ^b	0.130	0.001
Retention	5.08 ^a	2.29°	3.67 ^b	0.172	0.001
Ca retention/digested, %	75.5ª	49.2°	66.2 ^b	1.898	0.001
Mg balance, g/d					
Intake	5.48 ^a	5.81ª	5.60 ^a	0.1026	0.088
Feces	3.43 ^b	4.20^{a}	3.96 ^a	0.1001	0.001
Urine	0.93 ^b	1.29ª	1.05 ^b	0.0582	0.001
Retention	1.13ª	0.31°	0.58^{b}	0.045	0.001
Retention/digested, %	54.7ª	19.5°	35.3 ^b	1.87	0.001

Mean values in rows without a common superscript are different at p < 0.05

The output of calcium in the feces of the pigs fed the fresh taro diet was higher that for the pigs fed the control diet. This showed that a proportion of the calcium in the fresh taro leaf diet remained bound to oxalate and was not absorbed in the digestive tract. The reduction in the calcium output in the feces of the pigs fed the ensiled taro diet compared to the fresh taro leaf diet confirmed that the reduction of oxalates in the digestive tract had a positive effect on the absorption of calcium in this region. The overall retention of calcium by the pigs fed the three experimental diets followed the same trends observed in the fecal output from each diet.

The output of magnesium in the feces of the two taro leaf diets was higher (mean 19.0%) when compared to the control fish meal diet, which led to much lower overall retentions of magnesium by the pigs fed the two taro leaf diets. The retentions of calcium and magnesium were lower for the pigs fed the taro leaf diets when compared to the control fish meal diet. Ensiling the taro leaves improved calcium retention, from 2.29 to 3.67 g/day, and from, 0.31 to 0.58 g/day, for magnesium.

Discussion

Fish meal is widely used as an ingredient in diets for growing pigs because it contains a balanced pattern of essential amino acids required by the growing pig (Fowler 1997; Wang et al 2009). However, it is an expensive ingredient and many experiments have sought to reduce its content to lower the overall cost of pig diets. The substitution of some fish meal with plant-based protein has been researched extensively (An et al 2005; Ly et al 2011). The use of taro leaves as a protein source poses interesting problems that needs serious consideration because it is an abundant and cheap resource in central Vietnam. Adjustment of the diet to take account of its lower crude protein and higher crude fibre contents can provide a balanced diet but the total oxalate content of the taro leaves is still a significant problem. Fermentation of the taro leaves not only improved the retention of nitrogen, but also reduced the oxalate content. The total oxalate content of the row have 7800 mg/100 g DM, which was comparable to the values of seven different locally-grown cultivars of taro harvested earlier in the growing season (Hang et al 2016). Selection of different cultivars of taro could reduce the intake of total oxalate from the fresh leaves and would have a considerable effect on the overall outcome of a feeding experiment. Fermentation of the taro leaves led to a 75.3% reduction in the total oxs alate content (from 7800 to 1930 mg total oxalate/100 g DM), which had a significant effect on the retention of both calcium and magnesium by the pigs.

The lower ileal and total digestibility of the protein in the taro leaves (Table 3) compared with fish meal, could be attributed to the binding of some of the protein to the fibre in the taro leaves, reducing its digestibility(Kass et al 1980 ;Jorgensen et al 1996). Shayo and Udén (1999) reported that 64% of the protein in *Xanthosoma* leaves could be found in the neutral detergent fibre fraction; however, a much smaller fraction (21%) was reported by Leterme et al (2005) for protein in the fiber fraction of *Xanthosoma* leaves.

The digestibility coefficients of the total oxalates were very similar for all three diets, which confirmed the observations made by Hatch and Freel (2005) 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 (Liebman and Chai 1997; Liebman and Costa 2000), presumably the soluble oxalates chelated with calcium in the small intestine preventing its absorption. The higher digestibility coefficients for calcium and magnesium seen in pigs fed ensiled taro leaves compared to fresh leaves, may be the effect of ensiling reducing the pH from 7.1 to 3.6 (Hang et al 2016) in the silage as the lactic acid content increased after 14 days (from 0.78 to 1.52% (Hang et al 2016) of ensiling, and this would facilitate the digestibility and absorption of Ca and Mg. Weaver et al (1987) showed that calcium absorption in rats was decreased by oxalate was significantly lower when compared to vegetables with a low oxalate content (Heaney and Weaver 1990; Heaney et al 1988). These results suggested that calcium formed insoluble, non-absorbable complexes with soluble oxalates in the gastrointestinal tract. An important issue was the degree to which the content of soluble oxalate affects the availability of dietary calcium.

Most of the oxalate in the taro leaves and taro leaf silage in this experiment was insoluble oxalate (mean 60.5% of the total oxalate content, Table 1) and this oxalate fraction contained a large proportion of the total calcium content of the leaves. The calcium contained in the insoluble oxalate fraction was considered to be unavailable in animal feeds (Jiang et al 1996). Wang (1983) suggested that this problem could be solved by the fermentation occurring during the ensiling process.

In the present study, calcium and magnesium retentions were highest in the control fish meal diet; however, ensiling the taro leaves improved the digestibility of Ca and Mg and tended to improve the retention of N, Ca and Mg (Table 4). It was interesting to note that ensiling the taro leaves led to a 75.5% reduction in the total oxalate content of the ensiled taro leaves compared to the fresh taro leaves, but the proportion of insoluble oxalate remained the same (60.6%).

The volume of urine excreted by the pigs consuming high levels of ensiled taro foliage was markedly increased when compared to the output following the consumption of the control and fresh leaf diets. A similar observation was made by Giang et al (2010), who fed ensiled taro silage to growing pigs and showed that the volume of urine excreted increased markedly when ensiled taro foliage was fed at high levels. Presumably, this diuretic effect of the taro was caused by the need to excrete the soluble oxalate salts. There was also a difference in Mg retention between treatments with the result lowest in the fresh taro leaf diet (Table 3). Bohn et al (2004) showed, from experiments with humans, that the significant differences in Mg absorption were due to the higher oxalate content of spinach. An apparent increase in magnesium absorption, from 16 to 35%, has previously been observed in young pigs (Bartley et al 1961). These results confirmed that oxalic acid, which is one of the most highly oxidised organic compounds, was widely distributed in plants, and occurred as insoluble oxalates that were mainly bound to Ca²⁺ and Mg²⁺ ions (Holloway et al 1989; Savage et al 2000). The excess magnesium interacted with the calcium, leading to greater excretion and lower absorption of calcium (Gaál et al 2004. This experiment suggested that taro leaves with a relatively high total oxalate concentration could be considered as a useful ingredient in pig diets provided the leaves were ensiled.

Overall, this experiment showed that there was a significant relationship between oxalate intakes and the retention of calcium and magnesium, and that the fermentation of the taro leaves significantly improved the value of this resource for feeding pigs.

Conclusions

• Taro leaves are useful components of pig diets as they provide protein and carbohydrates, but the fresh leaves contain high levels of oxalates, which can substantially reduce the absorption of calcium and magnesium from the digestive tract.

- The oxalate contents of the ensiled taro leaves were considerably reduced when compared to the fresh taro leaves.
- This meant that the pigs consumed fewer oxalates and the overall digestibility of calcium and magnesium and the biological value of the protein were considerably improved when compared to the fresh taro diet.
- The overall nitrogen, calcium and magnesium retentions of the two taro leaf diets were still lower when compared to the control fishmeal diet.
- The oxalate content of taro leaves is a major factor to be considered when designing taro-based feeding systems for pigs.

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