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ORIGINAL PAPER

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The influence of oxalate decarboxylase on the urinary oxalate excretion in swine model of nephrocalcinosis induced by hydroxyproline

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ABSTRACT

Introduction. Kidney stone formation may be a result of increased urinary oxalate supersaturation.

Material and Methods. Eighteen pigs were randomly divided into: Control group, where standard cereal-based feed was supplemented with 4% HP only, Prevention group, where treatment with OxDc slurry started at the end of the adaptation period when pigs were switched to 4% HP diet, Reduction group, where the treatment with OxDc lyo powder started after pigs were already on a 4% HP diet for 6 days.

Results. OxDc slurry prevented oxalate excretion in urine. The reduction effect of OxDc lyo feed addition was generally visible during the first two days of the therapy ($p<0.05$). Both dietary intake of 4% HP and OxDc preparations did not influence weight gain, water or feed intake, urine excretion and creatinine clearance.

Conclusions. The capacity of OxDc in preventing induced hyperoxaluria was moderate. Most probably, this is due to the incoherent response of animals to the HP enriched diet dependent on their gut pH, since optimum pH for OxDc is around 5-6. A higher pH essentially reduces the activity of OxDc. The capacity of OxDc in reversing the hyperoxaluria induced by a HP enriched diet was significant during the first 2 days after introducing OxDc to the diet.

Keywords. Nephrocalcinosis, Oxalate decarboxylase, Hydroxyproline, Pig model

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Introduction

Kidney stone formation as a result of the accumulation of calcium salts in renal tissue is believed to be an effect of increased urinary supersaturation, mostly of calcium oxalate (CaOx) and calcium phosphate (CaP).[1] Therefore, lowering the concentration of oxalate and/or calcium in urine is an important part of medical treatment that could prevent the formation of calcium oxalate crystals and consequent crystaluria.

In healthy humans, urinary oxalate is derived from dietary (~40%) and endogenous pools (~60%). However, pathways leading to an endogenous synthesis of oxalate have not been fully elucidated. From studies in patients with type 1 and 2 of primary hyperoxaluria, high levels of oxalate are known to result from deficiencies in the glycolate metabolizing enzymes, alanine-glyoxylate aminotransferase and glycolate reductase.[2] Thus, glycolate is implicated as a precursor to endogenous oxalate. The endogenous precursors of glyoxylate and glycolate are not known to date, but hydroxyproline (HP) has been implicated as part of the pathway.[3]

The pig model of hyperoxaluria and calcium oxalate stone disease after feeding with hydroxyproline (HP) was described before by others.[4,5] HP, a component amino acid of collagen, is metabolized to pyruvate and glycolate, an oxalate precursor, in both hepatic mitochondria and renal proximal tubular cells.[3] It is important to address that at the functional level, humans and pigs/sows share many similarities with regard to the genitourinary structures. In addition to being multipyramidal structures (unlike rats), humans and swine have comparable maximal urinary concentration, glomerular filtration rate, and total renal blood flow characteristics.[4] Mandel NS et al. and Kaplon DM et al. [4,5] demonstrated that young growing pigs and sows fed HP became hyperoxaluric which provokes calcium oxalate plaque formation on renal papillae and stones known as crystalluria. Knight J et al. fed gelatin, a food ingredient that contains HP, to healthy human subjects, and created elevated levels of urinary oxalate and glycolate.[3] The Western-type diet, which has an abundance of animal protein, has been implicated as an increased risk factor for the formation of kidney stones in humans.[5] Consumption of the Western-type diet results in endogenous oxidation of excess cationic and sulfur-containing amino acids which impose a chronic metabolic acidosis that leads to bone demineralization and hypercalciuria that together with hyperoxaluria present a huge risk for stones and kidney failure.[5]

Oxalate decarboxylase (OxDc) is an enzyme that can be used for treatment of hyperoxaluria. OxDc has high specificity to degrade oxalate into the more soluble products carbon dioxide (CO_2) and formic acid (HCOOH). The rationale for the oral therapy with OxDc is that it is capable of breaking down oxalate found in the orally administered feed starting from the stomach and duodenum and thereby decreasing the oxalate available for absorption into systemic circulation. On the other hand, the decreased concentration of oxalate in the lower gut lumen will create a concentration gradient, causing the movement of oxalate back to the gut lumen from the blood, and enhancement of enteric excretion or intestinal elimination.[6] In the animal models, enteric excretion of oxalate is up regulated when the kidney function is compromised.[7]

The primary objective of this study was to find out if oral administration of OxDc will reduce urinary oxalate excretion and, by this means, will also prevent nephrocalcinosis and calcium oxalate crystal formation in kidneys of HP challenged pigs. The secondary objective was further development and testing of the swine model of dietary induced hyperoxaluria.

Materials and methods

All experimental procedures were approved by the Malmö/Lund Ethic Review Committee on Animal Experiments, Lunds city court (Malmö/Lunds djurförsöksetiska nämnd, Lunds tingsrätt), Lund, Sweden. During the study, the care and use of animals was conducted in accordance with the principles outlined in the current Guide to the Care and Use of Experimental Animals.

Animals and housing

The experiment was carried out on the Odarslövs research farm of Swedish Agriculture University and the Department of Biology of Lund University, Sweden.

Eighteen male pig (Swedish Landrace, Yorkshire, and Hampshire), 6 weeks of age and a weight of 6.5 ± 0.8 kg at the beginning of experiment, were used in the study. Animals were randomly selected from 7 litters from the University herd at Odarslöv, Swedish Agricultural University. The pigs had been weaned at four weeks of age and then housed in individual pens (1.0 × 1.5 m) with perforated plastic flooring, wood chips and bedding. All pens were equipped with a dry feeding trough, a drinking nipple and a constant heating lamp (150 W). During the experimental period, pigs were individually housed in "home-design" metabolic cages. Each metabolic cage was also equipped with a drinking nipple and red heating lamp (150 W). All pigs were acclimated and trained to the metabolic cage before the start of the experiment.

Study design

The eighteen pigs were randomly divided into three groups (n=6): 1) Control group, where feed was supplemented with 4% HP only, 2) Prevention group, where the treatment with OxDc slurry (3.5 mL, 15,750 u/meal) started at the end of the adaptation period when pigs were switched to 4% HP diet and 3) Reduction group,

where the treatment with OxDc lyo powder (500 mg, 11,500 u/meal) started after pigs were already for 6 days on a 4% HP diet. OxDc in form of slurry was given at the beginning of the meal with syringe to mouth to all pigs in the Prevention group, while OxDc lyo powder was mixed with the feed and was given with the first small portion of the meal to all pigs in the Reduction group. (Figure 1)

Monitoring and Assessments. Body weights were obtained before, during and at the end of experiments. Animals were also assessed for signs of poor health and other conditions that might interfere with results.

Feeding and water administration

Pigs were fed with cereal-based feed for young growing pigs (53908 VÄXTILL 320 P BK, Lantmannen, Sweden) twice daily (2% body mass per meal) at 8-9 am and 5-6 pm. This amount is comparable to the amount of consumed food when given *ad libitum* in similar conditions. During the pre-treatment period, feed was enriched gradually with HP (from 1% to 4%) and after a week of adaption all pigs were switched to a 4% HP diet until the end of the experiment. Drinking water was provided *ad libitum* when pigs stayed in pens, but during the study while pigs were in metabolic cages, water was provided 4 times per day, again *ad libitum*, for accurate measurement of water intake.

Inducing the hyperoxaluria. To achieve the hyperoxaluria, pigs were challenged with trans-4-hydroxy-L-proline (HP) obtained from TIANJIN WRI BR SHE YI SHU GOAN, BAN SHI CHU, 309 999 TIANJIN, China.

Oxalate decarboxylase

Oxalate decarboxylase used in the experiment was formulated as a slurry or lyophylisate (lyo) formulations. Lyo OxDc CLEC Lot #768-9 and slury OxDc Lot #768-9 (Altus Pharmaceuticals, Cambridge, MA, USA). Specific activity, of the both formulations were tested (Alnara Pharamceuticals, Cambridge, MA, USA) and the values were ~45 U/mg and ~23 U/mg for the OxDc slurry and lyo, respectively. Estimated activity of OxDc slurry might have been higher than indicated, due to improper dry weight measurement or increased solubility due to possible leaching of glutaraldehyde during storage. Both enzyme formulations were stored in tightly sealed containers at 2–8 °C.

Blood, urine and feces sampling

Blood and urine samples were taken at the following time points: basal (during adaptation time), last day on 2% HP and last day on 3% HP, before randomization, every second day during treatment period and on the last day of the study when pigs were sacrificed.

Blood. 5 mL blood samples were collected on the respective days before feeding from the jugular vein via direct venipuncture. Collected blood samples were placed in Vacutainer heparin tubes. After collection, tubes were centrifuged for 15 min at 3000 rpm and blood plasma was separated to new tubes. Samples were store at -20 °C for further analysis.

Urine. 24h urine samples were collected into container with 2 ml of 6 N HCL. After measurement of total volume of urine, 3 mL sample was transferred to the plastic tube and stored for further analysis.

Feces. Small fraction of fresh samples of the feces were collected in the morning from the pigs for oxalate estimation. Each sample was weighed, put in the plastic bag and stored at -20 °C for future analysis.

Analysis of oxalate concentration

Oxalate concentration in blood and urine was measured by sensitive spectrophotometric method with the use of Trinity Biotech Oxalate reagents (Kit #.591 D Trinity Biotech, Ireland). The severity of hyperoxaluria for the young growing pigs (5-12 weeks of age and 5-20 kg of body weight) was graded as shown in Table 1.

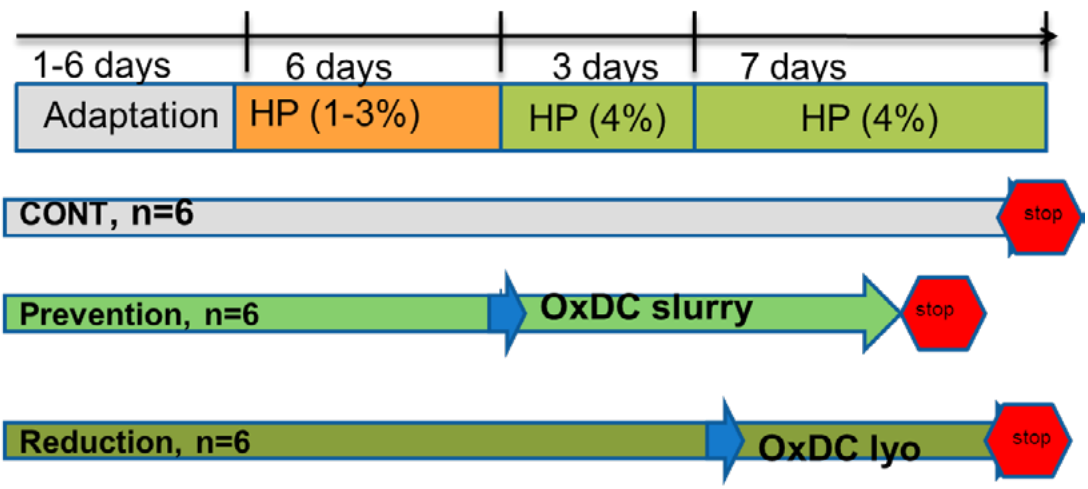


Figure 1. Study design

Table 1. Grading of the hyperoxaluria for the young growing pigs (5–12 weeks of age and 5–20 kg of body weight)

Hyperoxaluria	Urinary oxalate mg/24h
Normal	2–10
Minimal	10–15
Mild	15–40
Severe	>40

Analysis of creatinine concentration ant the creatinine clearence

Creatinine concentration. Creatinine in blood and urine was measured using a colorimetric method (QuantiChrom™ Creatinine Assay Kit, DICT-500, BioAssay Systems, USA). Urine samples that were used for analysis were acidified and charcoal extracted that might have resulted in lower read outs.

Creatinine clearance. Creatinine clearance is expressed as excretion rate ($U_{cr} \times V$), where U_{cr} presents concentration of creatinine (mg/dL) and V (Urine Volume) is the 24h urine sample (mL/24h), divided by plasma creatinine (Pcr in mg/dL), BW is body weight of pigs. The formula used was as follows:

$$C_{cr} = \frac{(U_{cr} \times \ Urine\ Vol\ xBW)}{P_{cr} \ \times 24 * 60} = mL/min$$

Collection of organs for histo-pathological analysis and gross examination

Gross analysis. At the end of the experiment, pigs were euthanized with sodium pentobarbiturate (20mg/kg). Selected organs: kidney, liver and small and large intestine were gross examined, kidneys and liver weight were recorded. Both right and left kidney were divided transversely longitudinally, exposing the corticomedullary surface and papillary tips. Gross appearance of the kidneys was recorded and digital images were obtained. After the gross examination, specimens of the kidneys, fixed in 10% formalin were taken for future histopathology analysis and Yasue specific staining.

Histopathology. Each kidney was cut in 12 serial sections at 4 μm per kidney and stained with hematoxylin and eosin for routine histological examination, or by specific Yasue metal substitution histochemical method to detect the presence of calcium oxalate crystals in the renal tissue.

Statistical analysis

Statistical analysis was performed on the data generated from this study using the unpaired two-tailed Student's t-test. Differences were considered significant if $p \leq 0.05$, all data are expressed as a mean ± standard deviation (±SD). The statistical software used was SAS *v* 9.2, 2008.

Results

Influence on the excretion of oxalate in urine

Total levels of oxalate in urine during pre-treatment and treatment period in both control and active groups were generally low and did not differ significantly ($p>0.05$). There was no effect of 4% HP administration on oxalate excretion in urine in both the Control and Prevention groups ($p>0.05$). Following, there was also no effect of oral administration of OxDc slurry visible in Prevention group ($p>0.05$). However, in the Reduction group, 4% HP induced severe hyperoxaluria (~5 fold elevation from normal levels) ($p<0.05$). The effect of OxDc lyo oral administration on oxalate excretion was visible from early stage of the therapy- as early as day 1 (6^{th} day from the beginning of study) ($p<0.05$). (Figure 2)

Influence on the renal function and urine excretion

Creatinine clearance. No differences in the creatinine clearance was noted between groups at baseline ($p>0.05$). Similarly, there was no difference recorded between the Control and the Prevention groups during the whole study period ($p>0.05$), which suggests also that there was also no differences in the GFR (*glomerular filtration rate*). However, creatinine clearance values were lowest, but not significant ($p>0.05$), in the Reduction group (which was in parallel with the highest values of oxalate excretion). Significant difference between the Control and the Reduction group (55.44 ± 31.38 mL/min *vs* 27.18 ± 19.93 mL/min, $p<0.05$) was recorded on day 22 (last day of the treatment). (Figure 3)

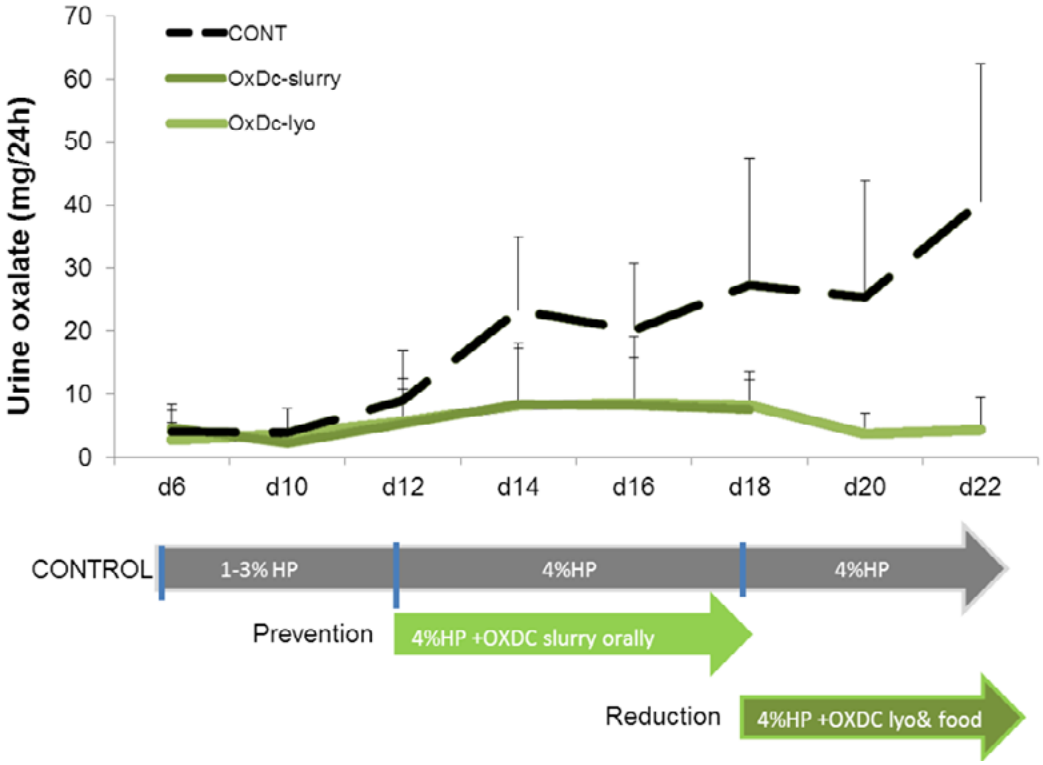
Urine excretion. There were distinct individual variations in both water intake and urine excretion within groups (data not shown). However, both water intake and urine excretion were higher in the treatment groups when compared to placebo, but the statistical significance was found only with regard to the Reduction group ($p<0.05$) (Table 2).

Table 2. Mean variations in feed intake (kg ± SD), water intake (kg ± SD) and the urine excretion (kg ± SD) during the whole study period. In all statistical analysis P<0.05 was taken as the level of significance

Groups	Food (g)	Water (mL)	Diuresis (mL)
CONT	276 ± 76.6	740 ± 229	278 ± 123
OxDc slurry	294 ± 64.39	903 ± 553	396 ± 327
OxDc lyo	336 ± 49.1	1150 ± 549	596 ± 463

Student's t-test: P<0.05

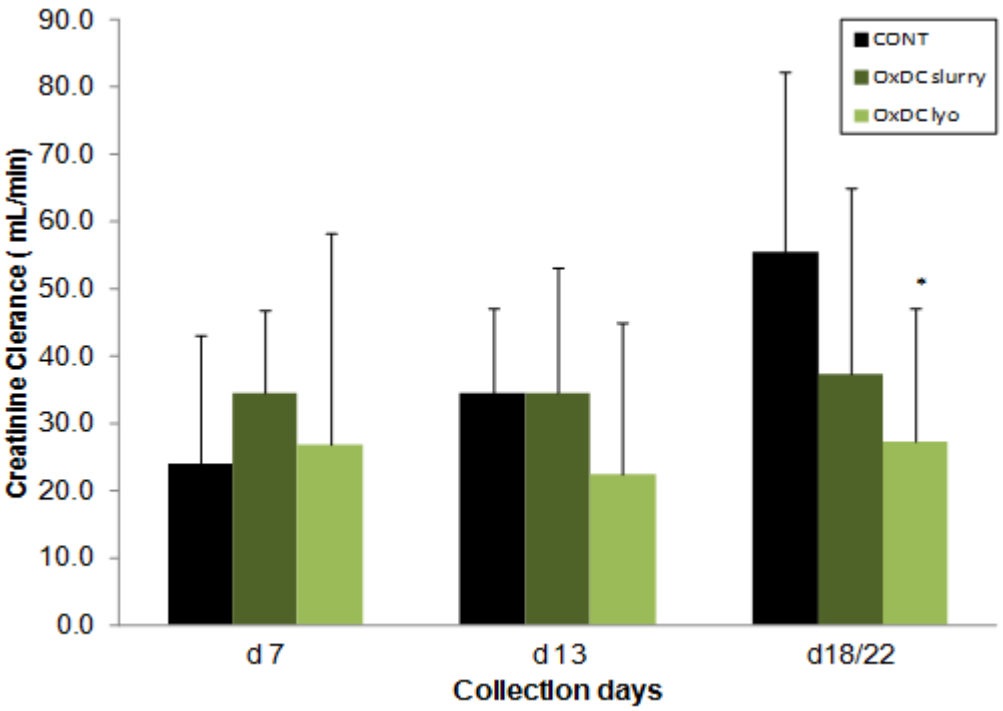
No statistical significance was found between study groups



Student's t-test: P<0.05

* Reduction vs Control group *P* < 0.05

Figure 2. Oxalate levels in 24h urine samples in pigs (mg/24h ± SD). In all statistical analysis, P<0.05 was taken as the level of significance



Student's t-test: P<0.05

* Reduction *vs* Control group *P* < 0.05

Figure 3. Creatinine clearance in pigs (mL/min ± SD). In all statistical analysis, P<0.05 was taken as the level of significance

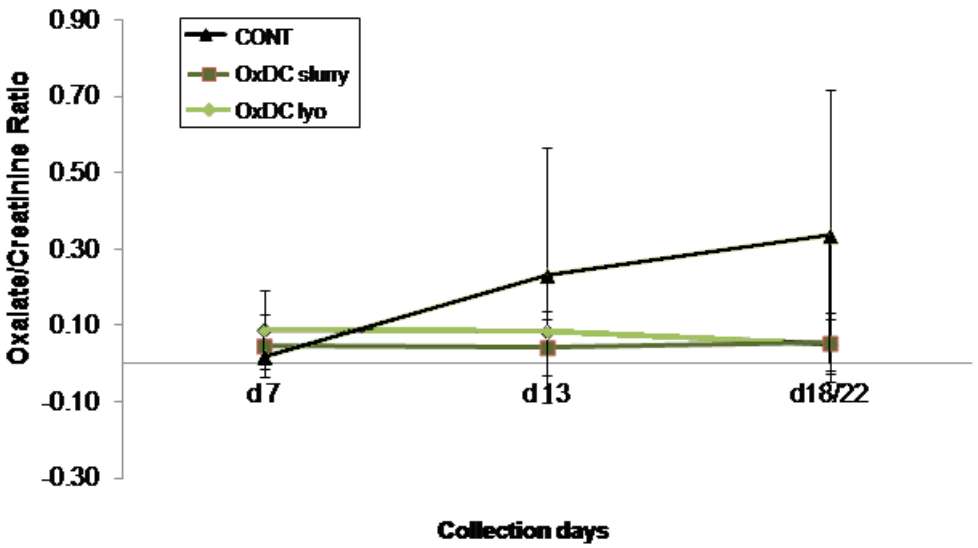
Influence on urinary oxalate levels normalized for daily creatinine excretion

Oxalate/Creatinine ratio. No differences in the oxalate levels normalized for daily creatinine excretion (oxalate/creatinine ratio) was noted within groups at baseline (p>0.05). Similarly, there was no difference recorded between the Control and the Prevention group during the whole study period (p>0.05). In the Reduction group, in which oxalate levels were increased, oxalate /creatinine ratio was also high and statistically significant when compared with Control group (p<0.05). (Figure 4)

Influence on body weight and feed consumption

Feed consumption. Daily feed consumption is presented in Figure 5. In general, differences in the daily feed consumption were observed in the selected time points within groups as well as between both treatment and control groups during the whole study period (p>0.05). (Figure 5, Table 2)

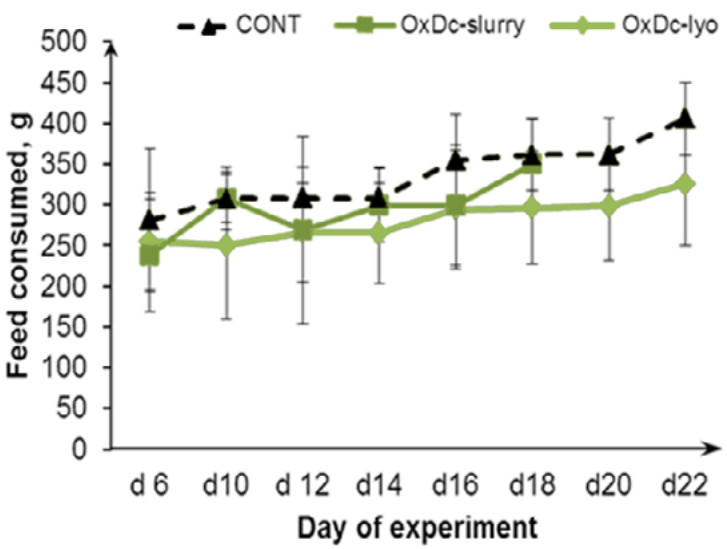
Body weight gain. There were no differences in body weight gain within groups as well as between experimental groups, however, the mean body weight in the Control group was insignificantly lower (~ 1 kg) when



Student's t-test: P<0.05

* Reduction vs Control group *P* < 0.05

Figure 4. Oxalate levels normalized for daily creatinine excretion in pigs (oxalate/creatinine ratio; L/min ± SD). In all statistical analysis P<0.05 was taken as the level of significance



Student's t-test: P<0.05

No statistical significance was found between study groups

Figure 5. Mean daily feed intake variation is study groups (g ± SD/24h). In all statistical analysis, P<0.05 was taken as the level of significance

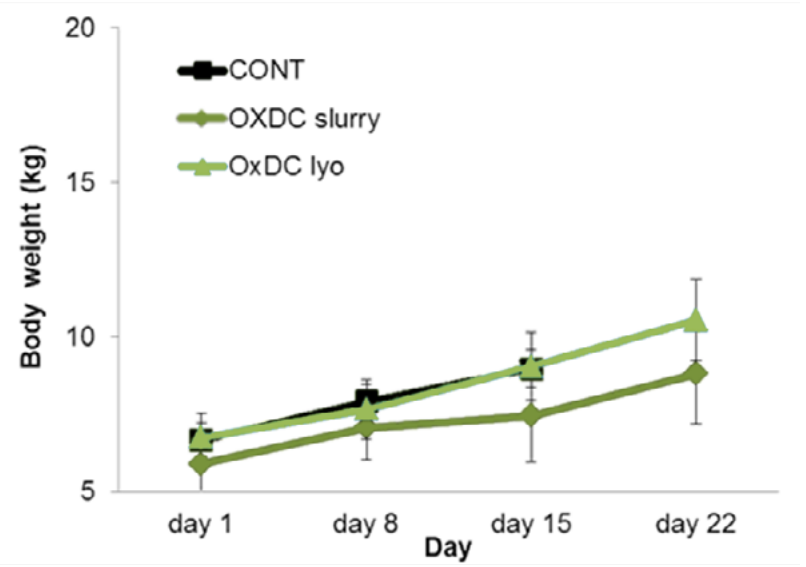
compared to both Prevention and Reduction groups at the end of the study (p>0.05). (Figure 6)

Macroscopic evaluation of the kidneys

The results of visual evaluation of the left and right kidney are given below.

Control group. In the control group. All six animals had fibrotic kidneys with the small spots of hemorrhages visible in both left and right kidney. Moreover in four of six animals in both sectioned kidneys small stones or/and crystals were visible.

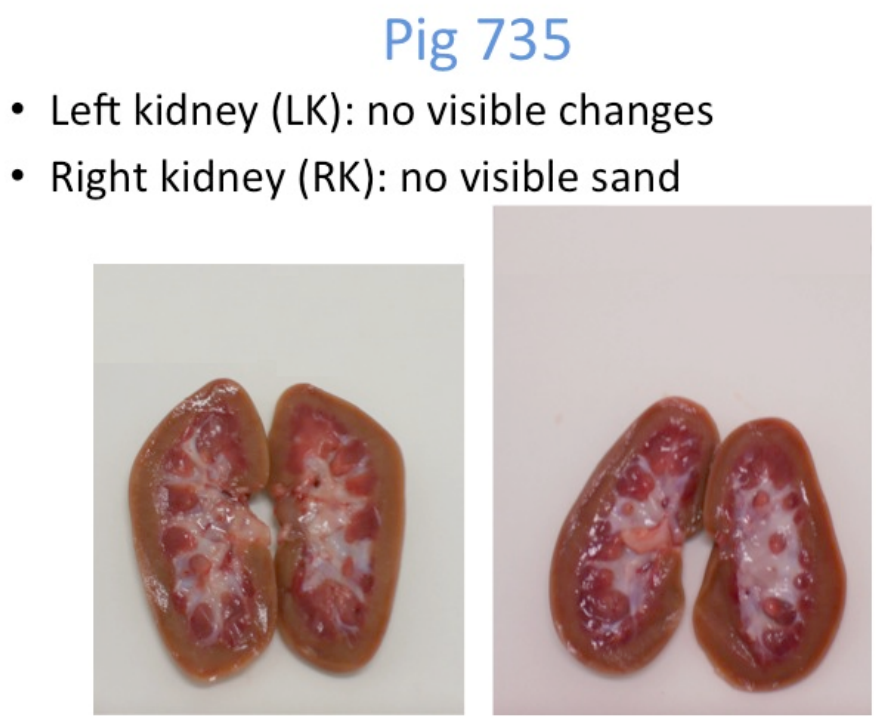
Prevention group. Two of six animals had normal kidneys, three had a small amount of crystals visible only in one kidney (left or right). One had small stones and crystals visible in both kidneys. (Figures 7a, 7b and 7c)



Student's t-test: P < 0.05

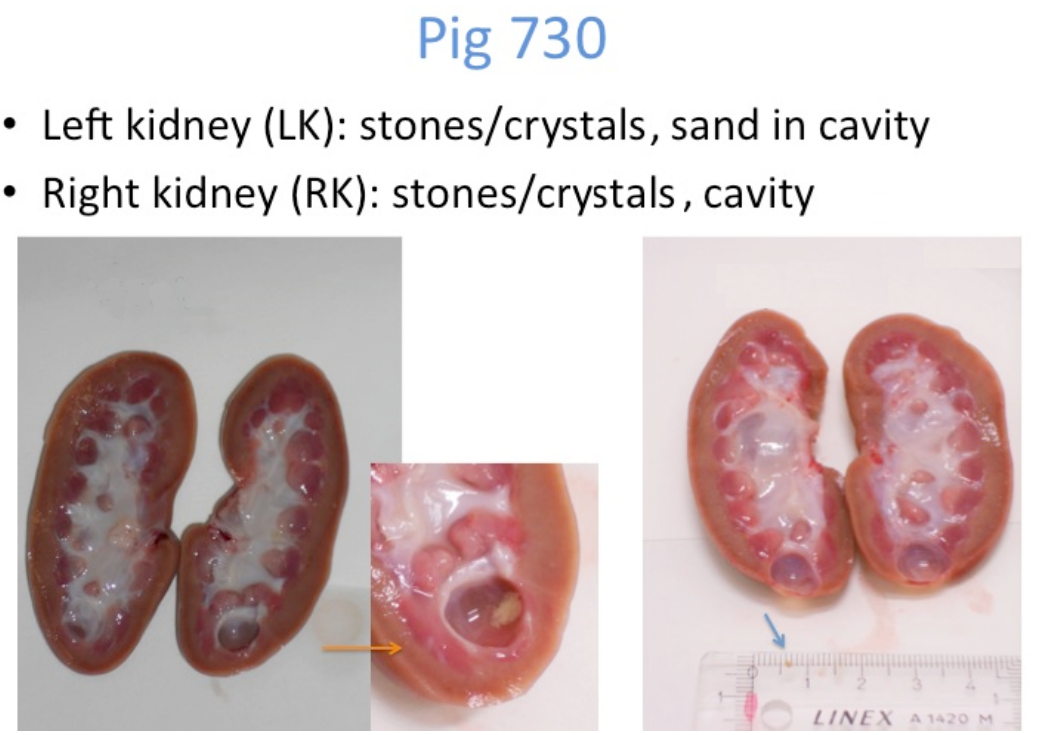
No statistical significance was found between study groups

Figure 6. Body weight during the pre-treatment and treatment phases (kg ± SD). In all statistical analysis P < 0.05 was taken as the level of significance

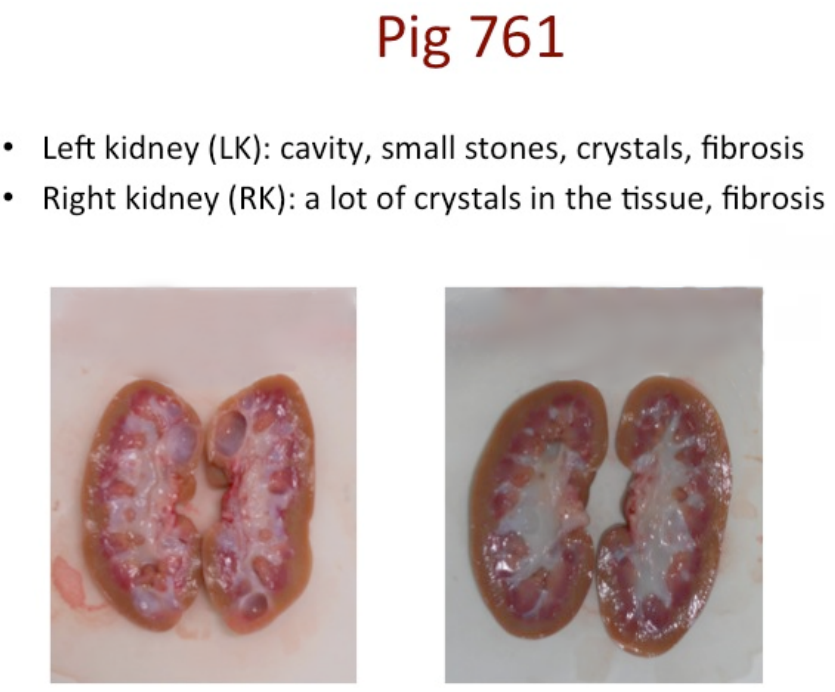


No macroscopic changes visible.

Figure 7a. An example of cross-sectioned normal kidneys (Prevention group)



Left and right kidneys: stones/crystals and abnormal cavities in medulla visible.
Figure 7b. An example of cross-sectioned kidneys with pathological changes (Prevention group)



Left and right kidneys: stones/crystals and fibrotic changes visible.
Figure 7c. An example of cross-sectioned kidneys with pathological changes (Prevention group)

Reduction group. Two of six animals had normal kidneys. Four animals had fibrotic kidneys with small cavities and small stones or/and crystals in visible in both left and right kidneys.

Histological evaluation of the kidneys

Thickness of the kidney cortex. No differences in the kidney cortex thickness was visible within groups (p>0.05). Similarly, there was no difference measured between the Control, Prevention and Reduction groups (p>0.05). (Table 3)

Table 3. Mean thickness of the kidney cortex (μm ± SD). In all statistical analysis P<0.05 was taken as the level of significance

Cortex thickness	Average
Control	4.68 ± 0.64
OxDc-slurry	4.75 ± 0.83
OxDc-lyo	4.86 ± 0.71

Student's t-test: P<0.05

No statistical significance was found between study groups

Histology examination using Yasue specific CaOx staining. CaOx stones were found mostly in the medulla of the kidney, suggesting that CaOx deposits are the result of oxalate precipitation during filtration through proximal and distal tubules. All animals in control group developed CaOx visible crystals in kidneys. Interestingly, in the group that was treated with OxDc-lyo (Reduction group) two out of six animals had normal kidneys without visible stones or crystals, while in the group treated with OxDc-slurry (Prevention) only one out of six pigs had normal (healthy) kidneys. (Table 4)

Discussion

based on the obtained results we believe that we have archived to develop a swine model of dietary induced primary hyperoxaluria. Most of the available data on kidney stone models concern rodents or piglets.[8,9] Although, our study is not the first presenting swine model of hyperoxaluria and stone formation, the novelty in the study was that we have archived the goal by administering to the pigs relatively low concentrations of HP in diet, when compared to other experiments.[1,4,5] This can be of great importance when taking under consideration the overall condition of animals. Modification of the diet by high dosing of HP or ethylene glycol may lead to appetite loss, lowering or gaining weight and may interfere the water intake and urine excretion.[10,11] Moreover, some data suggests that it may also cause metabolic acidosis and may lead to renal dysfunction.[10] All of the above mentioned dis-

Table 4. CaOx depositions in cortex (C), intermedulla (C-M) and medulla (M) of kidneys after staining with Yasue's metal substitution method that specifically detects calcium oxalate depositions*

Group	Pig #	Left kidney			Right kidney		
		C	C-M	M	C	C-M	M
CONT	727	none	none	minimal	none	none	minimal
	747	none	none	minimal	none	none	minimal
	757	none	none	none	none	none	minimal
	760	none	none	minimal	none	none	minimal
	765	minimal	minimal	minimal	none	minimal	moderate
	777	minimal	minimal	moderate	none	minimal	moderate
OxDc-slurry	724	none	none	minimal	none	none	minimal
	730	none	minimal	minimal	none	none	minimal
	735	none	minimal	minimal	none	minimal	minimal
	751	none	none	none	none	none	none
	759	none	none	minimal	none	none	minimal
	775	minimal	minimal	moderate	minimal	minimal	moderate
OxDc-lyo	731	minimal	minimal	moderate	minimal	minimal	moderate
	749	none	none	none	none	none	none
	754	none	none	none	none	none	none
	761	none	minimal	minimal	none	minimal	moderate
	763	minimal	minimal	minimal	minimal	minimal	moderate
	783	none	none	minimal	none	none	minimal

* For grading the severity of nephrocalcinosis , the scoring was done under a four category scale. The scoring was the following: none, no oxalate crystals in any field; minimal, 1-5 crystals in any field; moderate 6-10 crystals in any field; severe- all fields with multiple depositions of crystals.

crepancies may have an impact on the results of the studies with enzymes, drugs, etc., for which, in fact, animal kidney stone models are designed for, and, which is obviously of great importance for final outcome of studies. Of course, our newly developed model is not ideal, all pigs in control group and majority of animals in Reduction group had kidneys with clearly visible fibrotic changes, which raises the question about reliability and/or reversibility of this model. Further studies are needed to elucidate, at least in part, to what extend such way induced fibrotic changes may influence renal function.

In the presented study, supplementation of a typical diet with 4% HP did not influence weight gain, water or feed intake or urine excretion, creatinine clearance, and last but not least, the overall condition of the animals. However, we have surprisingly observed significantly lower oxalate excretion in the Control group when compared to the Reduction group both before and after the addiction the OxDc lyo to the diet. This is at least in part similar to the observations made by Kaplon et al., who observed, after a short peak in the first day of the oral 10% HP administration, a subsequent decline in urine oxalate levels despite continued feeding.[5] This is contrary to the results obtained by others as well as to our own results from previous experiments in which urine oxalate levels have significantly raised during the first few days and did not decline immediately after the HP enriched diet was ceased.[5,10] The explanation for this could be the individual differences in metabolic capacity and/or the influence of other, until now, unknown factors. But, we can be almost sure that the decline in oxalate urine levels observed in the Control group was not a result of the diet administration, because, as mentioned above, there were no differences in feed intake both within and between study groups. On the other hand, HP absorption in the gut and/or its subsequent metabolism to oxalate may have a rate limiting step, which was clearly shown by Bushinsy et al., who concluded that oxalate levels may not be strictly dose-related to HP in the diet.[11] Anyway, above described discrepancies raise also one more, very important issue regarding the action and effectiveness of OxDc, which in fact, was also the aim of the study.

Although the difference between the Control and Reduction group after 2 days of treatment (20th day of experiment) was found, it should not be attributed to increasing the oxaluria by 4% HP diet in Control animals, but rather to the decreasing oxaluria by the administration of OxDc lyo in the Reduction group. Moreover, the same issue does not allow us to conclude that the low oxaluria in the OxDc slurry receiving animals was really the result of enzyme action in the animal gut. However, we do not exclude such a possibility since macroscopic changes seen during the gross examination of the kidneys were much less advanced in animals receiving 4% HP enriched diet together with OxDc slury than in those receiving 4% HP enriched diet alone.

Both OxDc slurry and OxDc lyo were well tolerated and did influence water or feed intake as well as urine excretion and creatinine clearance. In the OxDc lyo receiving animals, we have observed a trend toward decreasing oxaluria during the first days of therapy (from the 18th until the 20th day of experiment), however, starting from day 21, urinary oxalate again increased reaching almost 40 mg/24h at the end of the study.

Another interesting observation from the presented study is that CaOx crystal/stone formation doesn't always correlate with the level of hyperoxaluria. Only the minimal urine oxalate excretion was recorded in the Control and Prevention group and yet, the majority of the animals had crystals or/and stones. On the other hand, in the Reduction group, where hyperoxaluria was relatively high, both gross and histopathological analysis did not show more pathology than we seen in other study groups.

Conclusions

In the presented experiment, we have created a model of hyperoxaluria in young pigs by increasing the amount of HP in the diet up to 4%. Histological and gross evaluation of the kidneys allows us to conclude that it is also a model for pig nephocalcinosis, due to clearly visible calcium oxalate crystals and small stones in renal tissue. The capacity of OxDc enzyme in preventing hyperoxaluria induced by HP enriched diet was moderate. This was most probably due to the incoherent response of study animals to the HP enriched diet dependent on their gut pH. It is well known that optimum pH for oxalate decarboxylase is around 5-6. Any higher pH essentially reduces the activity of oxalate decarboxylase. Anyway, the capacity of OxDc enzyme in reversing the hyperoxaluria induced by a HP enriched diet was significant during the first 2 days after introducing OxDc to the diet.

The model presented can be extrapolated to humans for future research. The rationale is that we nearly imitated the mechanisms which are, at least in part, responsible for the kidney stone formation in humans as in e.g. diet overloaded in animal protein and/or a highly acidifying diet. Moreover, pigs are believed to be the best model humans, due to very similar renal physiology and anatomical structure.

References

1. Khan SR. Nephrocalcinosis in animal models with and without stones. *Urol Res*. 2010;38(6):429-438.
2. Hope B, Kemper MJ, Bökenkamp A, et al. Plasma oxalate calcium supersaturation in children with primary

hyperoxaluria and end stage renal failure. *Kidney Int.* 1999;56(1):268-274.
3. Knight J, Jiang J, Assimos DG, Holmes RP. Hydroxyproline ingestion and urinary oxalate and glycolate excretion. *Kidney Int*. 2006;70(11):1929-1934.
4. Mendel NS, Henderson JR, Hung LY, Wille D, Wiessner JH. A porcine model of calcium oxalate kidney stone disease. *J Urol*. 2004;171(3):1301-1303.
5. Kaplon DM, Penniston KL, Darriet C, Crenshaw TD, Nakada SY. Hydroxyproline-induced hyperoxaluria using acidified and traditional diets in the porcine model. *J Endourol*. 2010;24(3):355-359.
6. Grujic D, Salido EC, Shenoy BC, et al. Hyperoxaluria is reduced and nephrocalcinosis prevented with an oxalate-degrading enzyme in mice with hyperoxaluria. *Am J Nephrol.* 2009;29(2):86-93.
7. Hatch M, Freel RW. Alterations in intestinal transport of oxalate in disease states. *Scanning Microsc*. 1995;9(4):1121-1126.
8. Mandel NS, Henderson JR, Hung LY, Wille D, Wiessner JH. A porcine model of calcium oxalate kidney stone disease. *J Urol.* 2004;171:1301-1303.
9. Khan SR, Glenton PA, Byer KJ. Modeling of hyperoxaluric calcium oxalate nephrolithiasis: Experimental induction of hyperoxaluria by hydroxy-L-proline. *Kidney Int*. 2006;70:914-923.
10. Goncharova K, Filip R, Świeboda P, et al. Model development of hydroxyproline induced hyperoxaluria in young growing pigs. *Eur J Clin Exp Med*. 2017;15(1):6-11.
11. Bushinsky DA, Asplin JR, Grynpas MD, et al. Calcium oxalate stone formation in genetic hypercalciuric stone forming rats. *Kidney Int*. 2002;61:975.