# CALCINOSIS IN FEMALE RABBITS

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## ABSTRACT

Soft tissue mineralization was determined in female rabbits on commercial farms. Does received vitamin $D_3$ supplementation subcutaneously, in drinking water, or both, at the beginning of every new reproductive cycle. Records (n=708) were obtained from on-farm necropsies of 245 dead and 463 euthanized rabbit does, sorted according to the age in months and the monthly extra dose of vitamin $D_3$ received. Data were gathered on 248 visits with necropsies on 101 doe farms in Spain, between August 2010 and December 2011. Producers administered extra doses of vitamin $D_3$ on 75% of the evaluated rabbitries; doses ranged between 1,000 and 125,000 IU per month. Median size of the 101 farms was 600 does (minimum to maximum: 90-7,000 does). The population at risk (in this study, females from 1st AI), was 190,481 does. Median age was 16 months (minimum to maximum: 5 to 59 months), and 6 kindlings (minimum to maximum: 1 to 34 kindlings), besides 57 females on their 1st pregnancy. Does with the most severe calcinosis were anorectic, emaciated (BCS=1/9), with extreme weight loss (BW= 2.2 kg), and died. At necropsy, 71 of 708 does (10%) presented variable scores of mineralization in the aorta, stomach, kidney, heart and other soft organs. The presence of calcium was confirmed on histopathological examination by von Kossa special stain. Calcinosis was assessed with the lesions in the aorta, on a 4-point linear scale, by trained and experienced veterinarians, according to images supporting this study. Using an ANOVA (proc CATMOD) we found that oral or mainly subcutaneous treatment with extra doses of vitamin $D_3$, was an enabling risk factor for calcinosis ($P< 0.0001$). The Odds ratio was 6.04 for >1,000 IU of vitamin $D_3$ per month, 11.40 for > 10,000 IU and 13.66 for > 25,000 IU. Age was a predisposing risk factor only in females receiving vitamin $D_3$ treatment, not in untreated does. Based on this retrospective study, we recommend the prudent use of vitamin $D_3$ in commercial rabbit production.

**Key words**: Animal welfare, calcinosis, diseases, rabbit, Vitamin $D_3$

## INTRODUCTION

Intestinal absorption of calcium in rabbits is proportional to the content of calcium in the diet, calcaemia being 2.17-4.19 mmol/L (Fudge, 2000). Rabbits excrete excess calcium via the urine, which predisposes calcification of soft tissues and urolithiasis (Kamphues, 1991); thus, when chronic renal failure occurs hypercalcaemia is very likely to occur too (Harcourt-Brown, 2007). Hypercalcaemia may also be the result of hypervitaminosis D, due to an excessive intake of vitamin $D_3$ or the ingestion of plants with the same level of toxicity as vitamin $D_3$ (Peixoto *et al.,* 2010). The reference plasma rate of 25(OH)$D_3$ is 250 nml/L; hypercalcaemia occurs when the concentration of 25(OH)$D_3$ or vitamin $D_3$ is > 375-500 nmol/L, approx. 150-200 ng/mL and 150-200 ng/d, respectively (Jones, 2008). Hypercalcaemia favours calcification of soft tissues. In rabbits, calcinosis has been associated with organ damage (Hass *et al.,* 1958, Hinton, 1981), and risk factors such as age and mineral content of

the diet (Nouaille *et al.*,1994), or a dietary overdose of vitamin D (Stevenson *et al.*, 1976). The aims of this retrospective study were to: 1) follow-up clinical cases due to vitamin $D_3$ toxicosis and 2) evaluate sporadic calcinosis in on-farm necropsies during 2010-2011.

## MATERIALS AND METHODS

### Farms and Rabbits

On August 2nd, we visited a farm housing 1000 rabbits to investigate the existence of underweight females, increased reproductive disorders and mortality in does. Toxicosis due to hypervitaminosis $D_3$ after subcutaneous administration of 125,000 IU of vitamin $D_3$ at each insemination (42-day cycle), was diagnosed. The same diagnosis and cause were found on another 5 farms until October 7th of the same year. The doses administered on all 6 farms were very high; however, between August 2nd, 2010 and December 31st, 2011, we evaluated calcinosis in samplings of 101 farms in Spain, on 248 visits during which we performed necropsies. The information was obtained from 708 necropsied does (serviced once or more); 245 were dead and 463 euthanized because they were moribund, or had been culled. 285 does were ≤ 12 months, 202 between 13 and 24 months, 134 between 25 and 36 months, 58 between 37 and 48 months and 29 between 49 and 59 months.

### Traits, laboratory analysis and statistical analysis

The method of euthanasia was concussion stunning, followed by neck (cervical) dislocation, plus exsanguination. During necropsy, the exterior and interior of the thoracic aorta and the heart, the kidneys and other organs in the thoracic and abdominal cavity were macroscopically examined. We based our classification of apparent causes of sickness or death on post-mortem macroscopic findings. We established 58 causes, including an "unknown case or cadaver with autolysis" and another "without any apparent lesions". One cause of mortality was calcinosis, which we evaluated according to the degree of calcification inside the thoracic aorta; we formed 4 groups: 0= with no apparent lesions, 1, 2 and 3, which was the maximum (Rosell, 2012). Other causes could be compatible with mortality, for instance, pneumonia, but we evaluated whether calcinosis had occurred simultaneously. On the farm visits, a record was made of the type of vitamin complex administered orally, parenterally or both. Also, the feed labels were checked for the content of vitamins, calcium and phosphorus declared and a record was made. During necropsy, samples were taken and put into 10% neutral buffered formalin and submitted for histopathologic processing in the laboratory; Von Kossa staining was used for diagnosis. Statistical analysis was performed by ANOVA for specific characters, following the SAS CATMOD procedure (version 9.1; SAS Inst., Inc., Cary, NC). The dependent variable in this model was calcinosis (YES/NO) and the independent ones were monthly dose of vitamin $D_3$ (×000 IU), with 5 levels: 0, 0-1, 1-10, 10-25 and >25 and age, with three levels: 5-12 m, 13-24 m, and > 24 m.

## RESULTS AND DISCUSSION

To study on-farm calcinosis in rabbit does, we performed 248 groups of necropsies on 101 farms with female rabbits, or females and bucks, on a total of 708 does. Artificial insemination (AI) was used on 87 % of the farms; 67 % of the females were serviced 11, 18 (12%), 25 (13%), or over 25 (8%) days later. Median size of the farms was 600 does (minimum to maximum: 90 to 7,000 does), with a population of 190,481 does at risk (inseminated at least once), during the 17 months of the study. Median age of the necropsied does was 16 months (minimum to maximum: 5 to 59 months), and 6 kindlings (minimum to maximum: 1 to 34 kindlings), besides 57 females on their 1st pregnancy. The extra dose vitamin $D_3$, administered parenterally, in the drinking water, or both, was given on 75 of the 101 farms, the doses ranging between 2,000 IU of vitamin $D_3$ and 125,000 IU, at each insemination. 479 does had been given extra doses of vitamin $D_3$; 158 does had been given no supplements at all. With regard to feed, the vitamin $D_3$, calcium and phosphorus contents were: 900-1,500 $IU.kg^{-1}$, as-fed, 0.85-1.4% and 0.43-0.72%, respectively; the vitamin $D_3$ levels were in line with current

recommendations: 3,000 IU $kg^{-1}$, maximum, which is considered harmless for does (Gidenne *et al*., 2010), <1,000 IU being the optimum content (Kamphues 1991).

With regard to the clinical examination, affected does were observed after the first pregnancy. They had been given between 25,000 IU and 125,000 IU of vitamin $D_3$; the first sign observed by the producers was anorexia, coinciding with descriptions by other authors (Stevenson *et al*., 1976, Löliger and Vogt, 1980). At the end of the first lactation, some females had received 250,000 IU of vitamin $D_3$ due to being inseminated twice. These females were emaciated; their body condition score (BCS) was 1/9, and the body weight (BW) 2.2-2.7 kg, although grade 3 does with BCS 6/9 and BW 4-5 kg were also weighed. Calcinosis was the only apparent disorder and therefore had a clear effect on BCS and BW, in accordance with preliminary observations (Sánchez *et al.,* 2012). On the second farm we visited, 50 % of the females were being given 125,000 IU of vitamin $D_3$ on the first IA, and the remaining 50% (groups of 90 females each) 2,000 IU. Mortality in the 2,000 IU groups was 5-7 % per batch (42 days), whereas in the groups injected with high doses, it was 12-16% for 42 days. Emaciated does that did not die on the 6 most affected farms were culled due to "bad body condition". Calcinosis also caused sterility (mount rejection or no pregnancies on AI farms), lower kit viability at birth and higher rates of abandoned litters. No signs of intercurrent diseases were observed on the first farm; in the sampling of 50 out of 320 lactating females, there was one case of coryza, 0 mastitis, 1 sore hocks and 1 mange.

In this study, there were 71 does with calcinosis, resulting in a 10% incidence risk, and 637 females not affected (90 %). There were 69 sick animals in the group of 479 treated females (14.4 %) and 2 in the non-treated ones (1.3%); also, 48 were score grade 3, 13 grade 2 and 10 grade 1. There were 53 females with lesions only compatible with calcinosis; 4 also had extrauterine pregnancies, 3 calcinosis and metritis, 3 nephropathies, 2 pneumonia, 2 with other obstetric causes, 1 with mastitis, 1 viral haemorrhagic disease, 1 hepatopathy and 1 mucoid enteropathy (similar to Epizootic Rabbit Enteropathy). Some females had grade 3 calcinosis, with no apparent lesions other than those in the aorta (Rosell, 2012), whereas in the most serious cases, lesions were observed in kidney, stomach serosa, mammary gland, abdominal musculature, heart and lungs. Zimmerman *et al*. (1990) found 33 affected rabbits aged 6 to 40 months, out of 278 treated ones (11.8%); in the present study, the highest number of sick females could have been due to higher doses of vitamin $D_3$ than those found by the mentioned authors. In our study, the presence of calcium was confirmed in the histopathology by von Kossa special stain.

Table 1 shows the ANOVA with 2 factors of variation: monthly dose of vitamin $D_3$, and the age of the does. Both variation factors were very significant $\chi^2 = 105.09$ ($P<0.0001$).

**Table 1**: ANOVA (proc CATMOD) for the calcinosis variable.

| Source | DF | $\chi^2$ | $P > \chi^2$ |
|---|---|---|---|
| Intercept | 1 | 140.50 | <0.0001 |
| Monthly dose of vitamin $D_3$ | 4 | 67.77 | <0.0001 |
| Age | 2 | 21.10 | <0.0001 |
| Likelihood ratio | 8 | 18.36 | 0.0187 |

The most important risk factor for calcinosis was the dose of vitamin $D_3$. Table 2 shows the effect of the level of vitamin $D_3$ on the frequency of does with calcinosis, the value of the $\chi^2$ of contingency was 106.14 ($P<0.0001$).

**Table 2**: Cross-tabulation level of vitamin $D_3$ and calcinosis.

| | Monthly dose of vitamin $D_3$ (×1,000 IU) | | | | |
|---|---|---|---|---|---|
| Calcinosis | 0 | 0.0-1.0 | 1.0-10.0 | 10.0-25.0 | >25.0 |
| No (n) | 158 | 109 | 181 | 170 | 19 |
| Yes (n) | 2 | 4 | 14 | 30 | 21 |
| Morbidity (%) | 1.3 | 3.5 | 7.2 | 15.0 | 52.5 |

The Odds ratio was 6.04 for females treated with >1,000 IU of vitamin $D_3$ per month, 11.4 for >10,000 IU and 13.66 for doses > 25,000 IU a month.

Regarding the age factor, although age was, on the whole, a significant factor in combination with the dose of vitamin $D_3$, in females not given an extra dose apart from the content in the feed, it was not a risk factor for calcinosis. Only 2 of the 71 affected does had not been given a vitamin $D_3$ supplement. Both of them were from a 76-day cycle farm. One was 48 months old, had grade 3 calcinosis, 17 parturitions and nephropathy; the other one was 56 months old, had grade 1 calcinosis, 19 parturitions but no apparent clinical diseases.

## CONCLUSIONS

The dose of vitamin $D_3$ administered orally in drinking water or via the subcutaneous route monthly, apart from the content in the feed, was a risk factor for calcinosis. The Odds ratio for does treated with over 1,000 IU a month was 6.04, 11.4 in those treated with > 10,000 IU and 13.66 in those treated with over 25,000 IU a month. Age was not a risk factor for calcinosis in females that did not receive an extra dose, but it was for those that did. Based on this study, we recommend prudent use of vitamin $D_3$ in commercial rabbit production.

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