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Calciotropic Principle of *Solanum glaucophyllum* in Broiler Chickens

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Abstract: *Solanum glaucophyllum* (duraznillo blanco) which causes calcinosis of cattle in Argentina, contains the active metabolite of vitamin D_3 either free or conjugated with carbohydrates as 1.25-$(OH)_2$ Vitamin D_3-glycoside. Economic relevance of *S. glaucophyllum* could be seen as being the causal factor of toxicosis of grazing cattle and also as a valuable source of Vitamin D_3 active metabolites. Some pharmacological applications in human and veterinary medicine as well as in animal husbandry have been assayed. The calciotropic principle was thought to be the main active metabolite of vitamin D_3, once liberated from its glycoside. However, calciotropic activity of this glycoside seems to be feasible. In order to know the fate of the water soluble calciotropic principle of *Solanum glaucophyllum* administered orally to broilers, 1,25-$(OH)_2$Vitamin D and 1.25-$(OH)_2$Vitamin D_3-glycoside plasma levels were assayed over a period of 24 h after a unique administration of the aqueous extract of the plant. Here we show that 1.25$(OH)_2$ vitamin D plasma levels did not increase while 1.25 $(OH)_2$ Vitamin D_3-glycoside rapidly appeared in blood, remained in circulation during the 1st 6 h and was undetectable 24 h after a unique oral administration of *S. glaucophyllum* (0.11 g DM kg^{-1} BW), to finishing broilers.

INTRODUCTION

Solanum Glaucophyllum (SG) Desfontaines (common name: duraznillo blanco) was first described in Brazil in 1846 under the name of *S. malacoxylon* Sendtner. In Argentina this species had been named *S. glaucum* Dunal/Bertolini but Cabrera showed that both names were conspecific (other names: *S. glaucescens* Baile and *S. glaucumfrutescens* Larranaga). An excellent description of the growth habit, the distribution and ecology of this weed in Argentina has been already published (Okada *et al*., 1977).

The ingestion of this plant, growing wild in South America and India (Deb, 1979), causes a calcinosis of cattle named Enteque Seco in Argentina and Uruguay and Espichamento in Brazil. The active principle associated with the pathological signs of this disease is the 1.25-$(OH)_2$ Vitamin D_3 (Haussler *et al*., 1976). This metabolite of Vitamin D_3 is encountered either free or conjugated with carbohydrates as 1.25-$(OH)_2$ Vitamin D_3-glycoside (Weissenberg *et al*., 1989).

Economic relevance of SG could be seen as being the causal factor of toxicosis of grazing cattle and also as a valuable source of Vitamin D_3 active metabolites. Some pharmacological applications in human and veterinary medicine as well as in animal husbandry have been assayed. Concerning human medicine, the main indications comprises chronic renal failure, hypo-parathyroidism and bone disorders. Some veterinary uses have been: the prevention of milk feber, pseudo-vitamin D deficiency of pigs and acidocis in chicks (Morris, 1982; Horst *et al*., 2003). The first animal husbandry application was the assay of the effect of SG leaves powder on egg shell quality in laying hens researchers concluded that there was a significative increase in egg shell thickness but a significative decrease in the percentage of laying. Intensive poultry production produces excess of P in manure. When this is applied on fields as fertiliser, the surplus can cause eutrophication of water bodies (Sharpley *et al*., 1994). SG has been added to the feed of chicks with the intention to improve phosphorus utilization and so, to reduce environmental phosphorus contamination due to poultry production (Cheng *et al*., 2004).

Additionally intensive broiler production also leads to higher occurence of skeletal problems and this in turn, to lower performance and animal welfare (Sanotra *et al*., 2001). A sequential study carried out on broilers suggested that the incidence of Tibial Dyschondroplasia (TD), one of the main skeletal problems was related to an inherent predisposition to rickets and to lower serum concentrations of 1.25-$(OH)_2$ D_3, throughout the period of maximums tibial growth (Parkinson and Thorp, 1996).

The calciotropic principle of SG was thought to be the main active metabolite of vitamin D_3, once liberated from its glycoside. The previous research done with rabbits, ewes (Dallorso *et al*., 2000) and cows (Dallorso *et al*., 2010) demonstrated early augmentation of 1.25-$(OH)_2$ Vitamin D plasma concentrations after a unique oral dose of aqueous extract of SG dry leaves. However this metabolite showed higher levels than untreated animals in plasma samples of rabbits administered with a unique subcutaneous dose of aqueous extract of SG dry leaves (Dallorso *et al*., 2000). The biological activity of 1.25$(OH)_2$ Vitamin D_3-glycoside was early demonstrated *in vitro* by Procsal *et al*. (1976) and more recently b y us when we were evaluating *S. glaucophyllum* toxicity *in vivo* with rabbits.

In order to know the fate of the water soluble calciotropic principle of SG administered orally to broilers, 1.25-$(OH)_2$ Vitamin D and 1.25-$(OH)_2$ Vitamin D_3-glycoside plasma levels were assayed over a period of 24 h after a unique administration of the aqueous extract of the plant.

MATERIALS AND METHODS

***Solanum Glaucophyllum* (SG):** Leaves of the plant collected in Cañuelas in *Buenos aires* province of Argentina in summer 2004 were dried, grounded and seived 1 mm (DM) and assayed by Radioimmunoassay (RIA) to evaluate its Vitamin D Activity (VDA; [1.25D] = 10 μg g^{-1} DM of SG) (Gil and Dallorso, 2002; Gil *et al*., 2007). The Aqueous Extract (AE) administered to broilers was prepared as in Dallorso *et al*. (2001) (0.33 g DM mL^{-1}; render of active principle 50%) and stored at -20°C in a dark bottle closed under N_2 until its administration.

Animals and treatments: About 28 males Cobb-500, 49 days old (mean Body Weight-BW) = 3.08±0.20 kg) from Granja Tres Arroyos, Capilla del Señor, Buenos Aires, Argentina were used. Birds were assigned at random to one of three groups. Group 1 (n = 12): received a unique oral dose of 1 mL of AE of SG (0.11 g DM kg^{-1} BW); Group 2 (n = 12): received a unique oral dose of 1 mL of run water; Group 3 (n = 4): birds of the same pen without treatment or manipulation. Animals were euthanized by complete bleeding 1 h (Group 1: n = 3; Group 2: n = 3), 3 h (Group 1: n = 3; Group 2: n = 3), 6 h (Group 1: n = 3; Group 2: n = 3) and 24 h (Group 1: n = 3; Group 2: n = 3), after each administration. Animals of Group 3 were euthanized at the beginning of the trial (time 0). Blood samples were heparinised to obtain plasma by centrifugation at 3500 rpm for 10 min, to be used for the determination of 1.25D and 1.25D_3-glycoside plasma concentrations by RIA.

Determinations: About 1 mL of plasma added with 0.050 mL ^{3}H-1,25D_3 (1000 cpm, ~170 Ci/mM) as recovery tracer+1 mL acetonitrile were vortexed and centrifuged at 4000 rpm for 15 min. Upernatant+0.5 mL of bidistilled water were applied to solid phase extraction minicolumns Waters SPE C18 (500 mg) that have been conditioned with hexane, cloroform, methanol and bidistilled water (Dallorso *et al*., 2001). The 1.25D_3-glycoside (F1) and 1.25D (F2) were eluted with 5 mL methanol:water (70:30) and 5 mL hexane: isopropanol (92:8), respectively. Both fractions were dried

in water bath bellow 40°C under N_2; F1 was resuspended in methanol: water (50:50) and F2 in ethanol, for RIA determination (Gil and Dallorso, 2002). The $1.25D_3$-glycoside concentration was measured against 1.25 $(OH)_2D_3$ (as standard of $1.25D_3$-glycoside is not available) and expresed as $1,25D_3$-glycoside equivalent to 1.25D in pg mL^{-1} plasma.

Analysis of data: About 1,25D plasma concentrations were examined by a 2-way ANOVA where one factor was TREATMENT (Grupos 1-2) and the other TIME (1- 3- 6 y 24 h). $1,25D_3$-glycoside plasma concentrations (Group 1) were studied by 1-way ANOVA where the factor was TIME (1-3-6 y 24 h). Comparsion of means were analysed by Tuckey. All the analysis were performed using the software Statistix SXW-Version 8.0.

RESULTS AND DISCUSSION

About 1.25D plasma levels at time 0 (Group 3) and 1-3-6 and 24 h after administrations (Groups 1 and 2) can be shown in Fig. 1. Basal mean value was 41.69±7.95 pg mL^{-1} (n = 4). Values did not differ neither between treatments nor among time after the administration. Nevertheless, we could see the lowest mean value (27.85±4.94 pg mL^{-1}; n = 3) 3 h after the administration of SG. Here we show that 1.25 $(OH)_2$ Vitamin D plasma levels did not increase after the administration of a unique oral dose of 0.11 g DM kg^{-1} BW of *S. glaucophyllum*, to finishing broilers. Other researchers have measured augmented plasma 1.25 $(OH)_2$ Vitamin D levels in birds treated with *S. glaucophyllum* previously incubated with β-glycosidases (Peterlik *et al*., 1976).

About 1.25D-glycoside plasma levels at time 0 (Group 3) and 1-3-6 and 24 h after the administration of SG (Group 1) can be shown in Fig. 2. Time 0 mean value was 0 pg mL^{-1} (n = 4). Values showed differences among time after the administration of SG by ANOVA (p = 0.05). The comparison of means showed significant differences by Tuckey (p = 0.1) of values corresponding to 1 h (18.08±4.83 pg mL^{-1}), 3 h (16.38±8.18 pg mL^{-1}) and 6 h (4.48±1.35 pg mL^{-1}) vs. those of 24 h (0 pg mL^{-1}), following the administration of SG. 1.25D-glycoside reached its peak 1 h and remained high up to 3 h, after the administration of SG while 1.25D showed its lowest plasma levels (Fig. 1 and 2). Note that the glycoside mass might be higher than expressed, here as displacement capability of 3H-1.25 $(OH)_2D_3$ from antibody binding sites by 1.25 $(OH)_2$ Vitamin D_3-glycoside has proven to be lower than that of the free metabolite of vitamin D_3 used as standard. In this research, plasma 1.25 $(OH)_2$ vitamin D_3-glycoside rapidly appeared in blood of broilers, remained in circulation during the 1st 6 h and was undetectable at 24 h, after a unique oral administration of *S. glaucophyllum*.

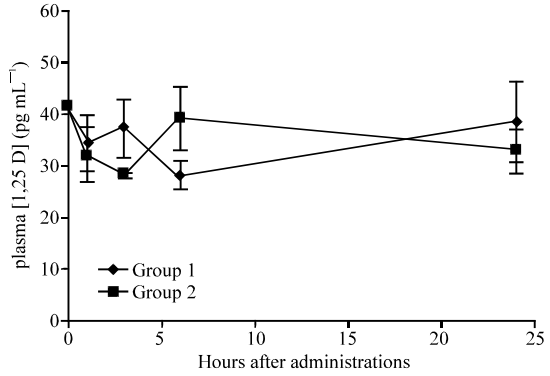

Fig. 1: 1.25 D plasma levels in male Cobb 500 finishing broilers after a unique administration of AE of SG (Group 1) or water (Group 2) by the oral route

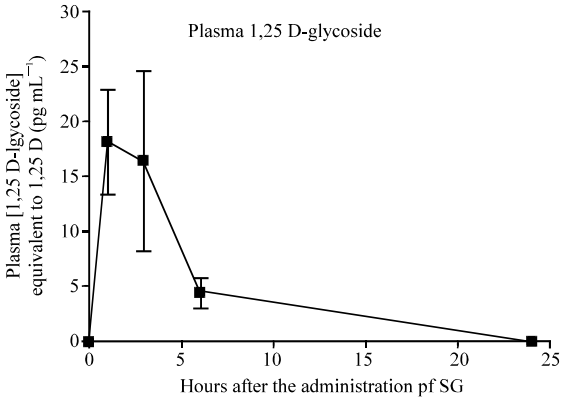

Fig. 2: 1.25 D-glycodide plasma levels in male Cobb 500 finishing broilers after a unique administration of AE of SG (Group 1) by the oral (Group 2) by the oral route

CONCLUSION

This is the 1 st time that 1.25 $(OH)_2$ Vitamin D and 1.25 $(OH)_2$ Vitamin D_3-glycoside were measured simultaneously in plasma of broiler chickens administered orally with *S. glaucophyllum*. The results obtained here are basic for those that are conducting feeding trails with *S. glaucophyllum* in poultry.

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