The effect of a new herbal vitamin D_3 on performance, blood parameters and tibial dyschondroplasia in broiler chickens.

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The purpose of this study was to evaluate a new vitamin D_3 of herbal origin (*Solanum glaucophyllum*) alone or in combination with cholecalciferol (VD_3) on performance, vitamin D-relevant blood parameters and tibial dyschondroplasia. The active compounds in *Solanum glaucophyllum* (PAN-HVD) have been identified as glycosides of 1,25-Dihydroxyvitamin D_3, the most active natural vitamin D metabolite in human and animal.

4900 chicken of hybrid Ross 308 were housed for final stocking density of 30 kg/m2. Animals were fed with a commercial broiler feed (Ca/P: 1.2) supplemented per kg with 25 µg cholecalciferol (A, control), 1 g PAN-HVD (B), 10g PAN-HVD (C), 1 g PAN-HVD plus 12.5 µg VD_3 (D) and 2g PAN-HVD plus 12.5 µg VD_3 (E) from day 1 to 38. Group C was included to examine the twelve-fold of the recommended dose.

Performance data of the trial was high with a low occurrence of tibial dyschondroplasia. Between treatments A, B, D and E no significant influences on performance could be observed. At day 38, mean scoring value of tibial dyschondroplasia was 0.58 (range 0-3). At day 14, plasma calcium was for the groups receiving PAN-HVD significantly higher (+10%; < p 0.05) than for the control group. All groups receiving PAN-HVD had higher plasma phosphate levels than controls: in tendency at day 14 and significantly (+27%; < p 0.05) at day 38.

After day 14 weight gain of group C decreased (-33%) and at day 25 half of the animals had depressed body weight. Plasma calcium (3.54 mmol/L) was 31% higher than of controls (2.68 mmol/L). The plasma concentration of 1,25-Dihydroxyvitamin D_3 was also elevated. All these findings indicate hypervitaminosis D. After cessation of treatment at day 25, the birds recovered within 14 days, weight gain and plasma calcium were normalized (2.78 mmol/l).

Administration of 1 g/kg feed of PAN-HVD resulted in equal performance and tibial dyschondroplasia scoring values as the control group. Slightly elevated plasma calcium and phosphate levels indicated a better mineral utilisation by PAN-HVD. In the animals fed twelve times the recommended dose retarded growth was observed, but recovered within few days after treatment was finished. Plasma calcium and phosphate levels as well as alkaline phosphatase activity seemed to be parameters to demonstrate an influence of vitamin D sources.

Keywords: Vitamin D_3; tibial dyschondroplasia; plasma calcium; plasma phosphate, Solanum glaucophyllum

Introduction

In poultry nutrition Vitamin D_3 is essential in regulation of the calcium-phosphor-metabolism; it enhances the resorption of Ca-ions in the intestinal tract and influences the bone metabolism (Scholtyssek S., 1987). The NRC (1994) recommends a minimum level of 200 IE (5µ/kg) of Vitamin D_3, whereas in Swiss commercial broiler diets 2000-4000 IE (50-100 µ/kg) of Vitamin D_3 are added.

The herbal Vitamin D_3 is produced from *Solanum glaucophyllum* (PAN-HVD, Herbonis AG, Basel, Switzerland). The active principles in this plant have been identified as glycosides of 1,25-Dihydroxyvitamin D_3, the most active natural vitamin D metabolite in human and animal (Soares et al., 1995 and Boland et al., 2003).

The effect of vitamin D_3 metabolites is often evaluated by measurement of parameters like tibial dyschondroplasia (TD), bone breaking strength and blood parameters. TD can lead to bone deformity and clinical lameness that still remain a welfare problem in intensive broiler production (Whitehead, 2004). It is well-know, that supplementation of Vitamin D metabolites are successful in prevention of TD (Edwards et al., 1992; Fritts and Waldroup, 2003) and in earlier studies with high dietary concentrations of vitamin D_3 up to 250µ/kg (Whitehead et al., 2004).

The purpose of this study was to evaluate a new vitamin D_3 of herbal origin alone or in combination with cholecalciferol on performance, vitamin D-relevant blood parameters and tibial dyschondroplasia.

Materials and methods

4900 Ross 308 broiler chicken were obtained from a commercial hatchery one day old and were put in place as hatched. The birds were housed in 20 floor pens (5 pens per treatment) on deep litter (straw pellets). At the end of the trial, stocking density was 30kg/m^2 as Swiss law allows. The animals were fed commercial broiler starter feed (crumbs, CP 200g/kg, ME 12.0MJ/kg), grower and finisher feed (pellets, CP 200 g/kg, ME 13.2 MJ/kg), which were formulated to a Ca: P-ratio of 1.2:1. A specific vitamin premix was added with adequate amounts of all vitamins except of vitamin D. Diets were supplemented either with the herbal vitamin D alone or in combination with synthetic vitamin D (*Table 1*). Treatment C was included to explore the safety margin of the herbal Vitamin D product by applying twelve-fold of the recommended dose.

Table 1 Supplementation of treatments with synthetic Vitamin D_3 (VD_3) and Herbonis Vitamin D_3 (PAN-HVD)

Treatment	VD_3 (µg / kg)	PAN-HVD (g/kg)
Control	25	-
B	-	1
C	-	10
D	12.5	1
E	12.5	2

Per pen live weights, feed consumption and mortality were recorded. Feed conversion was calculated as feed consumption divided by weight gain. At day 14 and 38, 20 resp. 10 birds per pen were selected and at 20 per treatment blood samples were taken at the jugular vain. Then these birds were culled. The growth plate of the right tibia was examined visually for incidence and severity of tibial dyschondroplasia on a scale from 0 (no) to 3 (gross abnormality) using a modified method of Sanotra et al., 2001. The blood samples were cooled and centrifuged. For analyses of plasma calcium, phosphate, alkaline phosphatase test-kits of Roche Diagnostics were used. Plasma 25-Hydroxyvitamin D_3 and 1.25-dihydroxyvitamin D_3 were analysed by using two ELISA-kits of Immunodiagnostik, Bensheim Germany.

Data were analysed by factorial analysis of variance (GLM-ANOVA) using the NCSS statistical package (Number Cruncher Statistical Systems, 2004). Significant differences between treatments were assessed by using the Bonferroni-test. Statements of significance were based on $p \leq 0,05$.

Results and discussion

Performance data of the trial, including the controls were high with low occurrence of tibial dyschondroplasia. Between treatments control, B, D and E no significant influences on performance could be observed (*Table 2*). But groups receiving PAN-HVD (B,D,E) had in tendency higher

performance than control. At day 38 mean scoring value of tibial dyschondroplasia was 0.58. There were no significant differences between treatments. TD-value of control was low with 0.53. This wasn't surprising because control feed contained 25 µg of Vitamin D_3 corresponding the fivefold of the minimal recommended dose. It is well known, that supplementations with vitamin D_3-metabolites (Fritts and Waldroup, 2003) or with high concentrations of vitamin D_3 (Whitehead et al., 2004) can prevent tibial dyschondroplasia. Furthermore complete prevention of TD was achieved with 1.25-dihydroxyvitamin D_3 (Rennie et al., 1993), the active component of *Solanum glaucophyllum* and thus also in PAN-HVD.

Table 2 Effect of PAN-HVD on live weight, feed consumption, feed conversion ratio (FCR), mortality and tibial dyschondroplasia (TD) at day 38 (TD also day 14)

Treatment	Control	B	D	E	Significance[1]	C [2]
Live weight (g)	2227	2261	2288	2230	n.s.	1322
Feed consumption (g)	3781	3863	3861	3750	n.s.	2222
FCR (kg feed/ kg weight gain)	1.728	1.739	1.717	1.713	n.s.	1.734
Mortality (%)	1.93	1.89	1.89	1.11	n.s.	7.43
TD day 14	0.36	0.32	0.29	0.22	n.s.	0.10
TD day 38	0.53	0.65	0.58	0.55	n.s.	0.50

[1] *=p<0.05,+=p<0.1, n.s.=not significant
[2] not included in statistical analyses

After day 14, live weights of group C were decreased by about –33 % compared to control (*Table 3*). At same time, plasma levels of calcium and 1,25-Dihydroxyvitamin D_3 were elevated. In the following days, birds of group C showed continuing depressed body weight and an elevated mortality rate, but without indication of a disease. At day 25, when half of the animals showed a reduced growth rate, an additional blood sampling was taken and the feed was replaced by the control feed. Plasma calcium of group C (3.54 mmol/L) was 31% higher than of controls (2.68 mmol/L). All these findings indicate hypervitaminosis D. After cessation of treatment at day 25, the birds recovered within 14 days, weight gain and plasma calcium were normalized (2.78 mmol/l).

Table 3 Development of body weight, cumulative mortality rate and plasma calcium (Ca++) of treatment C compared to control

	Day	Control	C
Live weights (g)	7	97	91
	14	474	317
	21	1121	601
Cumulative mortality (%)	7	0.61	0.61
	14	1.12	2.55
	21	1.56	3.89
Ca++ (mmol/l)	14	2.11	2.77
	25	2.68	3.54

At day 14, plasma calcium was significantly higher (+10%; < p 0.05) for the groups receiving PAN-HVD than for the control group (*Table 4*). All groups receiving PAN-HVD had higher plasma phosphate levels than controls: in tendency at day 14 and significantly (+27%; < p 0.05) at day 38. This could be an indication of a better utilisation of P and Ca in combination with PAN-HVD. Cheng et al., 2004, recorded that adding *Solanum glaucophyllum* to the diet of broilers can improve the efficiency of absorption and utilization of P and also Ca. The effect of PAN-HVD on utilisation of P and Ca could allow formulating broiler diets with lower P or Ca contents and thus a reduction of the P load in the manure.

Table 4 Effect of PAN-HVD on plasma calcium (Ca++), plasma phosphate (P), alkaline phoshpatase (ALP) 25-Hydroxyvitamin D_3 (CDL) and 1.25-dihydroxyvitamin D_3 (CTL) at day 14 and 38

	Treatment	Control	B	D	E	Significance[1]	C [2]
Day 14							
	Ca++ (mmol/l)	2.11^{b}	2.19^{ab}	2.35^{a}	2.33^{a}	*	2.77
	P (mmol/l)	1.69	2.00	1.98	1.90	+	0.99
	ALP (U/L)	27105	23451	24623	19557	n.s.	11928
	CDL (nmol/l)	1.28^{a}	0.11^{b}	0^{b}	0^{b}	*	0
	CTL (pmol/l)	264	295	211	366	n.s.	406
Day 38							
	Ca++ (mmol/l)	2.85^{a}	2.89^{a}	2.69^{b}	2.81^{ab}	*	2.78
	P (mmol/l)	2.37^{b}	2.70^{ab}	2.67^{ab}	3.27^{a}	*	2.76
	ALP (U/L)	3520	3584	4845	5499	+	4764
	CDL (nmol/l)	2.47^{a}	0.83^{ab}	0^{b}	0.23^{a}	*	6.70
	CTL (pmol/l)	225^{b}	328^{a}	301^{ab}	371^{a}	*	431

[1] *=$p<0.05$,+=$p<0.1$, n.s.=not significant
[2] not included in statistical analyses

Administration of 1 resp. 2 kg/t of Herbonis Herbal Vitamin D_3 to feed alone or in combination with cholecalciferol resulted in equal performance and tibial dyschondroplasia scoring values as the control group. Slightly elevated plasma calcium and phosphate levels indicated a better mineral utilisation by PAN-HVD. In the animals fed twelve times the recommended dose retarded growth was observed, but the animals recovered within few days after treatment was finished. Plasma calcium and phosphate levels as well as alkaline phosphatase activity seem to be valuable parameters to demonstrate the influence of vitamin D sources.

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