Safety profile of 1,25-dihydroxyvitamin D_3 of herbal origin in broiler chicken

G. Mathis[1], R. Boland[2], H. Bachmann[3], A. Toggenburger[1], W. Rambeck[4]

[1] Appletree CI Group AG, Winterthur, Switzerland, [2] INBIOSUR-CO, Universidad Nacional del Sur, Departamento de Biología, Bioquímica y Farmacia, Bahia Blanca, Argentina, [3] Herbonis AG, Basel, Switzerland, [4] Institut für Tierernährung, Ludwig-Maximilians-Universität München, Oberschleissheim, Germany

Summary

The safety of supplementing broiler feed with a standardised herbal extract, *Solanum Glaucophyllum Standardised Leaves* (SGSL) containing glycosylated 1α,25-dihydroxyvitamin D_3 (1,25$(OH)_2D_3$) and standardised to contain 10 µg/g 1,25$(OH)_2D_3$ equivalent, was examined in two studies. In a first study, we examined the potential of SGSL to substitute vitamin D_3 (VD_3) and the tolerated dose range of SGSL when applied without concomitant VD_3 by analyzing performance and blood chemical parameters after 14, 25 and 38 days on diets containing two doses of SGSL (1 and 10 g/kg feed) as source of 1,25$(OH)_2D_3$. In the second study, the no adverse effect level of SGSL was determined by analyzing the same parameters after 35 days on diets containing basic VD_3 supply and in addition 0.2, 1.0, 2.0 and 4.0 g of SGSL/kg feed. We showed that SGSL was able to substitute VD_3 in broilers as far as the performance parameters were concerned. Also, we found that the no adverse effect level is at least 4 g SGSL/kg feed when used with moderate doses of VD_3. This is 20 times higher than the upper limit of the commercially recommended dose. We concluded that SGSL is a safe feed additive to use in broiler chicken.

Keywords: 1,25-Dihydroxyvitamin D_3-glycosides, feed safety, *Solanum glaucophyllum*, Vitamin D, broiler

Sicherheitsprofil von 1α,25-Dihydroxyvitamin D_3 auf pflanzlicher Basis in Masthühnern

Die Sicherheit des standardisierten Pflanzenextrakts, *Solanum Glaucophyllum Standardised Leaves* (SGSL) als Futterzusatz bei Masthühnern wurde anhand von zwei Studien überprüft. SGSL enthält standardisiert 10 µg/g 1,25$(OH)_2D_3$ in glykosylierter Form. In der ersten Studie wurde das Potential, als Vitamin D_3 (VD_3)-Ersatz zu wirken und der tolerierte Dosisbereich von SGSL untersucht, indem SGSL ohne Zugabe von VD_3 gegeben wurde. Hierzu wurden Leistung und laborchemische Blutparameter nach 14, 25 und 38 Tagen Fütterung mit zwei unterschiedlichen Dosierungen SGSL (1 und 10 g/kg Futter) als 1,25$(OH)_2D_3$-Quelle untersucht. In der zweiten Studie wurde der No Adverse Effect Level anhand der gleichen Parameter nach 35 Tagen bei einer Fütterung, welche nebst einer moderaten Menge an VD_3 0.2, 1.0, 2.0 und 4.0 g SGSL/kg enthielt. Es konnte gezeigt werden, dass SGSL Vitamin D_3 bei Masthühnern ersetzen kann, was die untersuchten Leistungsparameter betrifft. Der No Adverse Effect Level lag bei mindestens 4 g SGSL/kg Futter wenn SGSL begleitend zu moderaten VD_3-Mengen gegeben wurde. Diese Dosis ist zwanzigmal höher als die kommerziell empfohlene Höchstdosis. Wir schliessen daraus, dass SGSL ein sicheres Futterzusatzmittel bei Masthühnern ist.

Schlüsselwörter: 1,25-Dihydroxyvitamin D_3-Glycoside, Futtersicherheit, *Solanum glaucophyllum*, Vitamin D, Masthühner

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Introduction

Vitamin D_3 (VD_3) per se is biologically inactive. It requires two metabolic conversion steps to obtain the active metabolite, 1,25-dihydroxyvitamin D_3 (1,25$(OH)_2D_3$), which is the key regulator of calcium homeostasis and phosphorus utilization (Feldmann, 2011; Bikle, 2014). The first metabolic step occurs in the liver, resulting in 25-hydroxyvitamin D_3 (25(OH)D_3), which is bound to a vitamin D-binding protein in circulating blood. In a second hydroxylation step, which is catalysed in the kidneys, the active metabolite, 1,25$(OH)_2D_3$, is formed. 1,25$(OH)_2D_3$ is rapidly eliminated from the system by the enzyme vitamin D_3-24-hydroxylase. The blood concentration of both VD_3 metabolites is similar in most species (Horst and Littledike, 1982). As a rule, the concentration of 25(OH)D_3 is in the nanomolar range, while the concentration of 1,25$(OH)_2D_3$ is on the picomolar level, approximately 2 orders of magnitude lower. An experiment by Goff and Horst (1995) demonstrated the homeostatic regulation of 1,25$(OH)_2D_3$ concentrations by adding increasing amounts of VD_3 to chicken and measuring the blood concentration of the metabolites 25(OH)D_3, 1,25$(OH)_2D_3$ and 24,25-dihydroxyvitamin D_3 (24,25$(OH)_2D_3$). The experiment showed a dose-dependent increase of the liver metabolite 25(OH)D_3, the levelling off of the concentration of the active metabolite 1,25$(OH)_2D_3$ above a supplementation level of 25 µg/kg feed, and the concomitant increase of 24,25$(OH)_2D_3$ concentrations. The experiment also shows the homeostatically regulated plateau of plasma calcium of around 2.6 mmol/L above a dose of 5 µg VD_3 per kg feed. As expected, bone calcium increased over the whole VD_3 dose range (Fig. 1). Although in nature, vitamin D is formed in the skin under UV irradiation (Kühn et al., 2015), in modern animal farming VD_3 is generally supplemented to feed. In particular, in commercial poultry meat production, addition of high doses of VD_3 (up to the currently authorised maximum dietary content of 125 µg/kg feed) is necessary to ensure optimal bone development and prevention of leg weakness (Whitehead et al., 2004). Experimental work by different groups found that 1,25$(OH)_2D_3$ was more potent than VD_3 and 25(OH)D_3 in preventing leg weakness, such as tibial dyschondroplasia, in fast growing broiler chicken (Edwards, 1990; Rennie et al., 1995; Edwards, 2002).

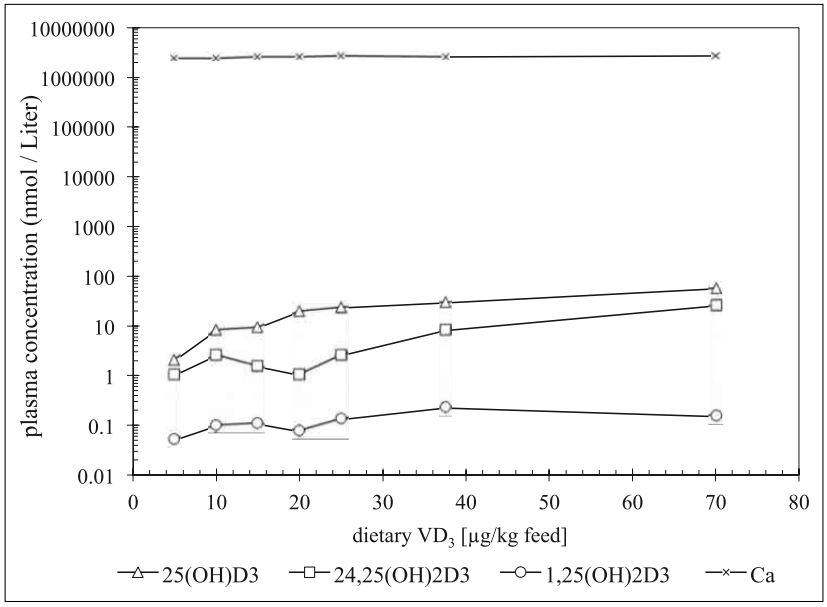

Figure 1: Plasma vitamin D metabolite concentrations after feeding increasing amounts of vitamin D_3 to chicken on a purified VD_3-free diet. Data reproduced with permission from Goff and Horst, 1995.

Recently, a 1,25$(OH)_2D_3$-containing standardised herbal product, *Solanum glaucophyllum Standardised Leaves* (SGSL), derived from the plant *Solanum glaucophyllum* (formerly named *Solanum malacoxylon*), has become available as a supplement for animal nutrition (Bachmann et al., 2013). The plant's pharmacological effects were discovered due to the disease *Enteque Seco* in the Lowlands of Argentina with cattle showing toxic signs after ingesting the plant in an uncontrolled manner. The similarity of the symptoms to hypercalcaemia led different research groups to the identification of the active component as 1,25$(OH)_2D_3$-glycosides (Napoli et al., 1977; Weissenberg 1989; Boland et al., 2003). It has been shown (de Boland et al., 1978; Boland et al., 1987; Mello and Habermehl, 1992; Skliar et al., 1992) that the 1,25$(OH)_2D_3$-glycosides from *Solanum glaucophyllum* are cleaved in the intestinal tract by ubiquitous glycosidase enzymes, and that subsequently the free aglycon enters the body. The release of 1,25$(OH)_2D_3$ from the glycosides upon intestinal passage is thought to be the rate limiting step resulting in a slow release pharmacokinetic profile when compared with synthetic 1,25$(OH)_2D_3$, which shows immediate release characteristics (Bachmann at al., 2013). The optimal, empirically determined dose recommendation by the supplier is to supplement feed with 0.05 to 0.2 g/kg SGSL (corresponding to 0.5 to 2 µg/kg analytically determined 1,25$(OH)_2D_3$ equivalent) on top of the usual concentrations of VD_3 contained in commercial feed. The safety of feed supplementation with 1,25$(OH)_2D_3$ has been questioned because relevant steps for the regulation of calcium homeostasis are bypassed. Namely, the risk of increased incidences of hypercalcaemia and associated soft tissue calcification has been discussed (Coburn and Maung, 2003; Towler, 2011). As a consequence we have evaluated the risk profile of feed supplementation with 1,25$(OH)_2D_3$ with a focus on the plant-derived glycosylated form.

Animals, Material and Methods

Two safety studies in broiler chicken were performed using *Solanum glaucophyllum Standardised Leaves* (SGSL, Panbonis®, supplied by Herbonis Animal Health GmbH, CH-4302 Augst, Switzerland). SGSL is standardised to contain 10 µg/g $1,25(OH)_2D_3$ equivalent. The composition of SGSL is shown in Table 1.

Table 1: Composition of *Solanum Glaucophyllum Standardised Leaves* (SGSL).

Water	7–10%
Crude ash	10–12%
Crude protein	21–23%
Crude fat	3–4%
Crude fibers	10–11%
Total carbohydrates	41–43% (Reducing sugars: 7–8%; starch 2–3%)
Alkaloids	<0.1%

Study I, substitution and dose range

This field study had the dual objectives to a) evaluate the potential of SGSL to substitute VD_3 and b) determine the maximally tolerated dose of SGSL for the subsequent study with respect to adverse effects. 2'940 Ross 308 broiler chicken *as hatched* were housed for 38 days in 3 treatment groups of 4 pens with 245 animals each at Aviforum (Swiss Institute for Poultry Research, Zollikofen). The animals had free access to water and a commercial pelleted maize, soymeal and wheat starter as well as grower diet devoid of vitamin D_3 (Provimi-Kliba, Kaiseraugst; Tab. 2). For the control group, the basic feed was supplemented with 25 µg VD_3 per kg feed (considered a moderate, but sufficient substitution) and for the two treatment groups with 1.0 and 10 g of SGSL per kg feed (corresponding to 10 and 100 µg $1,25(OH)_2D_3$/kg feed, respectively), doses exceeding the upper limit of the commercially recommended dose by a factor of 5 and 50, respectively.

Table 2: Analysis of nutrients of the starter, grower and finisher diet in study I.

	Unit	Starter	Grower	Finisher
Dry matter	%	89	88	89
Crude ash	g/kg	51	48	47
Crude protein	g/kg	212	230	238
Crude fibers	g/kg	25	22	21
Crude fat	g/kg	57	76	78
Carbohydrates	g/kg	49	48	50
Starch	g/kg	403	370	369
AME	MJ/kg	12.6	13.0	13.2
Ca	g/kg	8.91	8.25	7.37
P	g/kg	7.36	7.10	6.94

AME: apparent metabolisable energy

The animals were daily monitored for health and mortality, and performance data were recorded at day 0, 14, 28 and at study end (day 38). The performance parameters were expressed as body weight (BW) in g; body weight gain at end of trial (BWG) in g; European broiler index (EBI), calculated as:

EBI = daily weight gain [g] × (100 – mortality [%])/ 10 × FCR

where FCR = feed conversion ratio = feed intake [kg]/ body weight gain [kg].

Blood samples were drawn in lithium heparin tubes from the wing vein of randomly selected animals per group on day 14 (20 animals), day 25 (10 animals) and day 38 (20 animals). Plasma was prepared by centrifugation (2000 rpm, 10 minutes) and stored at –20 °C until calcium (Ca), phosphorus (P), $25(OH)D_3$ and $1,25(OH)_2D_3$ were analysed. Plasma calcium and phosphorus were determined using commercial test kits from Roche Diagnostics (#11730240, o-kresolphtalein method, and #11730347, ammonium phosphor molybdate method, respectively, Roche Diagnostics AG Switzerland). Plasma alkaline phosphatase was determined with a test kit from Roche Diagnostics (# 2173107, p-nitrophenyl phosphate method, Roche Diagnostics AG Switzerland). Concentrations of 25-Hydroxyvitamin D and $1,25(OH)_2D_3$ were determined with commercial ELISA kits (#K2110 and #K2112, respectively, ImmunDiagnostik AG, Bensheim, Germany).

The test diet was to be withdrawn and the animals were to be switched to control diet if the average blood calcium level exceeded 3 mmol/L, the conventionally accepted level indicating the lower limit of hypercalcaemia. The study was performed under Swiss Animal Welfare Law (authorization by Amt für Landwirtschaft, Bern, authorisation number 35/05)

Statistical analysis was performed by using unifactorial analysis of variance using the program NCSS 2004 (Statistical Systems, Kayville, UT, USA). Significant differences were accepted if $p < 0.05$. In the event of significant differences, the Bonferroni test was applied.

Study II, No Adverse Effect Dose

This laboratory study had the objective to determine the no adverse effect dose of SGSL under conditions imitating commercial use, i.e. adding increasing amounts of SGSL (0.2, 1.0, 2.0 or 4.0 g per kg feed corresponding to 2, 10, 20 and 40 µg $1,25(OH)_2D_3$ / kg feed, respectively) to a usual VD_3-containig diet (80 µg/kg feed). The lowest dose of SGSL in this study (0.2 g/kg feed)

corresponded to the upper limit of the commercially recommended SGSL supplementation dose. The highest dose (20-fold higher) was chosen to a) meaningfully exceed the upper limit of the commercially recommended dose and b) to remain well below the non-tolerated dose of 10 g/kg feed (see results of study I). 690 male Ross 308 broiler chicken were housed for 35 days in 5 treatment groups of 6 pens each with 23 animals each at Schothorst Feed Research, NL-Lelystad. The animals had free access to water and a commercial pelleted maize, soymeal and wheat diet (Arkervaart-Twente, NL-Leusden; Tab. 3). The study followed the Technical Guidance for Tolerance and Efficacy Studies in Target Animals published by European Food Safety Authority (EFSA, 2011) and was approved by the Animal Ethical Review Committee and was carried out under code SFR-2012-31 according to the restrictions provided under the Animal and Human Welfare Code of the Netherlands. All 5 groups (including the control group) received a starter diet (days 1–10), a grower diet (days 11–25) and a finisher diet (days 26-35), containing a basic vitamin D_3 supply of 80 µg/kg feed. The treatment groups received SGSL in addition to the basic diet.

The animals were monitored for health and mortality and performance data were recorded. The parameters were expressed as: body weight (BW) in g; body weight gain at end of trial (BWG) in g; European broiler index (EBI) using the same formula as in study I.

On day 35 blood was drawn from the wing vein and collected in EDTA tubes for haematological analyses (white blood cells (WBC), red blood cells (RBC), haemoglobin, packed cell volume), mean corpuscular volume and into serum tubes for clinical chemical and enzymatic analyses (calcium (Ca), phosphorus (P), magnesium (Mg), alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase (ALP), creatine phosphokinase and $1,25(OH)_2D_3$). Blood and serum analyses were made using commercial analyzers. After blood sampling, the animals were euthanised by intracardiac injection of T61 (Intervet International BV, Boxmeer, The Netherlands) and 12 animals per treatment group were examined for gross pathology, which included heart, respiratory system, digestive tract, liver, kidneys, spleen, bursa, bones, muscles and joints. The following organ samples were collected: kidney, duodenum, heart, liver, tibia, a piece of aorta and a 1-cm transversal segment of the proventriculus. One kidney per animal was put in formalin, as was the slice of the proventriculus and the aorta sample, the other kidney was cooled on ice. The duodenum was rinsed with PBS and also put on ice, as were heart and liver. All cooled samples were stored at –20 °C until shipment and further analysis. Formalin samples were stored at room temperature. Left and right tibias were collected separately and stored at –20 °C. Calcium deposition was examined in formalin–fixed, paraffin-embedded kidney, aorta and proventriculum samples using the von Kossa staining method (Sheehan and Hrapchak, 1980). Collected left tibias were autoclaved as pooled sample per treatment group during 30 minutes at 120 °C to remove residual meat. The clean bones were weighed, defatted and ashed for 18h at 700 °C, and then weighed again. Tibia ash was expressed in g/kg total fat-free tibia dry matter. Statistical analyses were done with GenStat® for Windows Version 15 (VSN International Ltd, Hemel Hempstead, UK) using least significant difference for treatment means. Production performance was analysed by regression analysis and analysis of variance. Significant differences were accepted if $p < 0.05$.

Table 3: Analysis of nutrients of the starter, grower and finisher diet in study II.

Nutrients	unit	starter	grower	finisher
Moisture	g/kg	119	120	123
Crude ash	g/kg	57	49	46
Crude protein	g/kg	219	195	190
Crude fat	g/kg	57	70	81
Crude fiber	g/kg	27	30	30
AME	MJ/kg	11.93	12.35	12.67
Ca	g/kg	6.5	5.0	4.5
P	g/kg	5.8	5.0	4.7

AME: apparent metabolisable energy

Results

Study I, a field study under commercial conditions, was designed to determine the effect of substitution of VD_3 with SGSL at exponentially different doses (1 and 10 g SGSL/kg feed corresponding to 10 and 100 µg $1,25(OH)_2D_3$/kg feed, respectively). The animals receiving 10 µg of $1,25(OH)_2D_3$ equivalent/kg feed performed as well as the control group (Tab. 4), thus demonstrating at least equivalent efficacy between 1g SGSL per kg feed and 25 µg VD_3 per kg feed as far as body weight, European Broiler Index and mortality were concerned. In this group, plasma $1,25(OH)_2D_3$ at day 38 was elevated when compared to control values, demonstrating a measurable increase in plasma $1,25(OH)_2D_3$ due to feed supplementation with SGSL. The elevated $1,25(OH)_2D_3$ did not, however, lead to increased calcium concentration in plasma suggesting that calcium homeostasis is not negatively affected by SGSL supplementation at this dosage.

In the animals supplemented with 100 µg of $1,25(OH)_2D_3$ equivalent/kg feed (Tab. 4), growth retardation was evident already at day 14 and blood calcium was elevated (+30% over control), one of the lead symptoms of a hypervitaminosis D. At day 25, blood calcium was almost

Table 4: Study I. Performance and plasma chemistry in broiler chicken when *Solanum Glaucophyllum Standardised Leaves* (SGSL) was used as a substitute to Vitamin D_3 (VD_3). Chickens received different SGSL diets and were compared to a control group receiving a VD_3 containing diet during 38 days. The SGSL 10 and SGSL 100 diet was devoid of VD_3. Nine hundred eighty animals were included in each group.

	Assessment	Unit	Day	Control VD_3 N=980	SGSL10 N=980	SGSL100 N=980
Diet	VD_3	µg/kg		25	None	none
	$1,25(OH)_2D_3$	µg/kg		none	10	100
Performance	Body weight	g	14	403	408	323
			25	1393	1416	989#
			38	2227	2261	1322#
	European Broiler Index		14	198	199	178#
			25	307	321	307
			38	327	330	181#
	Mortality	%	14	1.12	1.02	2.55
			25	1.67	1.44	6.74
			38	1.93	1.89	7.43#
Plasma chemistry	Ca ± SD	mmol/L	14	2.11 ± 0.07	2.19 ± 0.24	2.77 ± 0.38
			25	2.68 ± 0.44	nd	3.54 ± 0.62 #
			38	2.85 ±0.23	2.89 ± 0.14	2.78 ± 0.17
	P ± SD	mmol/L	14	1.69 ± 0.49	2.00 ± 0.31	0.99 ± 0.43
			25	2.65 ± 0.81	nd	2.07 ± 0.53
			38	2.37 ± 0.75	2.70 ± 0.48	2.76 ± 0.7
	$25(OH)D_3$ ± SD	mmol/L	14	3.2 ± 3.275	0.275 ± 0.85	0#
			25	nd	0	0
			38	6.175 ± 4.85	2.075 ± 4.60	16.75 ±10.13#
	$1,25(OH)_2D_3$ ± SD	nmol/L	14	0.634 ±0.086	0.708 ± 0.492	0.974 ± 0.456
			25	1.039 ± 0.137	1.039 ± nc	1.426 ± 0.137
			38	0.540 ± 0.089	0.787 ± 0.122 #	1.034 ± 0.144 #

Control VD_3, basic diet containing 25 µg VD_3 per kg feed; SGSL10, basic diet without VD_3 containing 1 g SGSL/kg feed (corresponding to 10 µg $1,25(OH)_2D_3$ per kg feed); SGSL100, basic diet without VD_3 containing 10 g SGSL/kg feed (corresponding to 100 µg $1,25(OH)_2D_3$ per kg feed); SD, standard deviation; nd, not determined; nc: not calculated; # statistically significant difference ($p < 0.05$) to control.

70% higher than control reaching 3.54 mmol/L and accumulated mortality rose from 1.67% to 6.74%. At this point, the animals were switched to the control diet containing only VD_3. As a consequence, at study end blood calcium had returned to normal and blood $1,25(OH)_2D_3$ was reduced by almost 30% compared to day 25.

Essential parameters of study II are summarised in Table 5. The animals reached an average final body weight of 2750 g at day 35. The performance parameters outperformed the breeder's performance curve by 500g (expected average 2250 g according to Aviagen, 2012) and were for all treatment groups marginally, but statistically significantly better than control.

The haematological parameters were in all treatment groups within normal range and gross pathological examination did not show any treatment-related effects (data not shown). Similarly, no clinically relevant effects on enzymatic activities in serum were found (data not shown with the exception of ALP, Tab. 5). Serum calcium was SGSL dose-dependently elevated without reaching critical (≥ 3mmol/L) levels, reflecting the expected effect of $1,25(OH)_2D_3$ on calcium uptake. Concentrations of Phosphorous were also increasing. Magnesium, the homeostasis of which is not considered to be VD_3-dependent, did not change. Plasma $25(OH)D_3$ was, unexpectedly, almost dose-dependently increasing.

No soft tissue calcification was found in kidney, aorta or proventriculus. Tibia bone ash was normal in all treatment groups, indicating absence of calcium mobilization from bone tissue (data not shown).

Discussion

Study I showed that SGSL at a dose of 1 mg/kg feed (corresponding to 10 µg $1,25(OH)_22D_3$/kg feed) was able to substitute VD_3 in broilers as far as the perfor-

Table 5: Study II. Effect of *Solanum Glaucophyllum Standardised Leaves* (SGSL) used as addition to a VD_3- containing diet on the performance, plasma chemistry and enzymology compared to a control group receiving a Vitamin D_3 containing diet. Assessments were done on day 35.

	Treatment group (N = 138 animals per group)	units	Control	SGSL2	SGSL10	SGSL20	SGSL40
Diet	VD_3	µg/kg	80	80	80	80	80
	SGSL	g/kg	0	0.2	1.0	2.0	4.0
	Corresponding to 1, 25$(OH)_2D_3$	µg/kg	0	2	10	20	40
Performance	Body weight	g	2669	2733 #	2698 #	2681 #	2746 #
	European Broiler Index		363	372	381	367	377
	Mortality	%	7	7	5	6	5
Plasma chemistry / enzymology	Ca	mmol/L	2.66	2.78 #	2.79 #	2.88 #	2.91 #
	P	mmol/L	2.49	2.52 #	2.33 #	2.57 #	2.58 #
	Mg	mmol/L	1.06	1.11	1.01	1.05	1.05
	Plasma 25$(OH)D_3$	nmol/L	190	210	210	220	230
	Plasma 1,25$(OH)_2D_3$	nmol/L	0.324	0.307	0.293	0.324	0.345
	Alkaline Phosphatase	units/L	11.51	12.80	11.94	13.18	10.72

VD_3, group of broiler chickens receiving control diet containing 80 µg Vitamin D_3 (VD_3) per kg feed; SGSL2, receiving control diet supplemented with 0.2g SGSL/kg, corresponding to 2µg 1,25$(OH)_2D_3$ per kg feed: SGSL10 receiving control diet supplemented with 1g SGSL/kg, corresponding to 10µg 1,25$(OH)_2D_3$ per kg feed: SGSL20 receiving control diet supplemented with 2g SGSL/kg, corresponding to 20µg 1,25$(OH)_2D_3$ per kg feed: SGSL40 receiving control diet supplemented with 4g SGSL/kg, corresponding to 40µg 1,25$(OH)_2D_3$ per kg feed; # statistically significant difference ($p < 0.05$) to control.

mance parameters examined are concerned. This conclusion is corroborated by the fact that the animals receiving SGSL had very low levels of 25$(OH)D_3$, which is evidence for the absence of VD_3 in the diet. Levels of 25$(OH)D_3$ rose markedly after the broiler chicken previously receiving the high dose SGSL (10g/ kg feed) were switched to the control diet, when the VD_3 contained therein was metabolised to 25$(OH)D_3$ by liver enzymes.

SGSL (1 g/kg feed) at a dose corresponding to 10 µg 1,25$(OH)_2D_3$/kg feed was well tolerated whereas a diet supplemented by SGSL (10 g/kg feed) corresponding to 100 µg 1,25$(OH)_2D_3$/kg feed resulted in clear symptoms of hypervitaminosis D as evident in the clinically manifest growth retardation and increased mortality concomitant with hypercalcaemia.

As a consequence, in study II, a standard broiler diet containing VD_3 was supplemented with increasing doses of SGSL up to 4 g/kg feed. Under these conditions, normal performance parameters, no pathological plasma values and no adverse effects were detected in any of the test groups. Neither calcinosis nor clinically relevant calcium or phosphorus mobilization from bone occurred, and ALP, an enzyme involved in phosphate deposition during bone mineralization, remained unchanged suggesting that calcium homeostasis is not affected by SGSL supplementation at the present dose levels. The body weight of the animals receiving the SGSL supplemented diet showed a statistically significant difference to control. However, this effect cannot be considered clinically relevant. Perhaps the overall better performance of all groups when compared to the breeder performance curve masked potential clinically relevant growth promoting effect of SGSL. Importantly, the highest supplementation level of 4 g SGSL/kg feed exceeded the commercial recommendation by a factor of 20. Consequently, the no-adverse effect level of SGSL under commercial conditions can be concluded to be at least 4 g of SGSL/kg feed corresponding to 40 µg of 1,25$(OH)_2D_3$ equivalents/kg feed. This is at least 4-fold higher than the no-adverse effect level for synthetic 1,25$(OH)_2D_3$, which was found to cause growth depression as the first observable adverse event from 10 µg/kg feed (Aburto et al., 1998). The observed, markedly better safety profile of SGSL when compared with synthetic 1,25$(OH)_2D_3$ may be explained by the delayed release characteristics of 1,25$(OH)_2D_3$ from SGSL resulting in a lower plasma peak concentration and a longer half-life when compared to its synthetic counterpart (Mathis et al., 2014). It may be speculated that with lower peak concentrations, SGSL derived 1,25$(OH)_2D_3$ remains below the threshold level inducing adverse effects.

In summary, *Solanum glaucophyllum Standardised Leaves* is a safe feed additive to use in broiler chicken up to a dose of 4g/kg feed, and can be used in combination with standard concentrations of VD_3.

Acknowledgement

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Profil de sécurité de la 1,25-dihydroxyvitamine D_3 d'origine végétale chez les poulets d'engraissement

Dans la cadre de deux études, on a examiné la sécurité de l'extrait de plante standardisé *Solanum Glaucophyllum* Standardised Leaves (SGSL) comme complément alimentaire chez les poulets d'engraissement. Le SGSL contient de façon standardisée 10 µg/g de $1,25(OH)_2D_3$ sous forme glycolysée. Dans la première étude, on a examiné le potentiel d'action en tant que remplaçant de la vitamine D_3 (VD_3) et le domaine de dose de SGSL toléré, ceci en ne donnant que du SGSL sans addition de VD_3 . On a examiné la performance et les paramètres de chimie sanguine après 14, 25 et 38 jours d'affouragement de deux doses différentes (1 et 10 g/kg d'aliment) de SGSL comme source de $1,25(OH)_2D_3$. Dans la seconde étude, on a recherché le *No Adverse Effect Level* sur la base des mêmes paramètres après 35 jours avec une alimentation contenant, outre une quantité modérée de VD_3, 0.2, 1.0, 2.0 et 4.0 g de SGSL/kg. On a pu démontrer que le SGSL peut remplacer la vitamine D_3 chez les poulets d'engraissement en ce qui concerne les performances étudiées. Le No Adverse Effect Level se situait aux environs d'au moins 4g de SGSL/kg d'aliment lorsqu'il était associé avec des quantités modérées de Vitamine D_3. Cette dose est vingt fois supérieure à la dose maximale recommandée par le fabriquant. Nous en déduisons que le SGSL est un complément alimentaire sûr pour les poulets d'engraissement.

Profilo di sicurezza dell'1,25-diidrossivitamina D_3 a base vegetale nei polli da carne

Si è voluto dimostrare, sulla base di due studi, la sicurezza dell'estratto vegetale standardizzato delle foglie di Solanum glaucophyllum (*Solanum Glaucophyllum Standardised Leaves* SGSL) come additivo per mangimi per polli da carne. SGSL contiene 10 µg/g di $1,25\ (OH)_2D_3$ sotto forma glicosilata standardizzata. Nel primo studio, è stato esaminato il potenziale della sua azione come sostituto della vitamina D_3 (VD_3) e l'intervallo della dose tollerata di SGSL, nel quale SGSL è stato incorporato senza aggiungere VD_3. A questo scopo le prestazioni e i parametri biochimici del sangue sono stati esaminati dopo 14, 25 e 38 giorni di alimentazione con due dosaggi diversi di SGSL (1 e 10 g/kg di mangime) come $1,25\ (OH)_2D_3$. Nel secondo studio è stata esaminata la dose priva di effetto avverso (NOAEL), sulla base dei medesimi parametri, dopo 35 giorni con un'alimentazione che assieme ad una moderata quantità di VD_3 0.2, 1.0, 2.0 contiene 4.0g SGSL/kg. È stato dimostrato che la vitamina D_3 di SGSL può essere sostituita nei polli da carne, per quanto riguarda i parametri di performance studiati. La dose priva di affetto avverso si situava al minimo a 4g SGSL/kg di mangime quando SGSL veniva somministrato in concomitanza con moderate quantità di VD_3. Questa dose è di venti volte superiore alla dose massima raccomandata in commercio. Concludiamo che SGSL è un additivo alimentare sicuro per i polli da carne.

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Corresponding author

Georg Mathis
Appletree CI Group AG
Rudolf-Diesel-Strasse 3
CH-8400 Winterthur
Switzerland
Tel: +41 52 209 06 40
Fax: +41 52 209 06 50
E-Mail: g.mathis@appletree-cig.com