

## Vitamin D<sub>3</sub> sources: 25-hydroxy vitamin D<sub>3</sub> as an alternative to the traditional vitamin D<sub>3</sub> source

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### Abstract

Vitamin D<sub>3</sub> is added to pig feed in the form of cholecalciferol. For the pig to be able to utilize this form of vitamin D<sub>3</sub>, it must be transformed into 25-hydroxy vitamin D<sub>3</sub> (HYD) via the liver. The addition of HYD will save the pig one step in the transformation process from vitamin D<sub>3</sub> to HYD in the liver. HYD is the form of vitamin D<sub>3</sub> that has the greatest biological value to the pig. The standard for vitamin D<sub>3</sub> to pigs is stated in international units (i.u.) per FUGp, but can also be converted into microgram: 40 i.u. vitamin D<sub>3</sub> equals 1 µg vitamin D<sub>3</sub>. In Denmark, the allowed maximum addition per kg complete pig diet is 2000 i.u. (=50 µg) total vitamin D<sub>3</sub>. The sum of these two vitamin D<sub>3</sub> sources is called total vitamin D<sub>3</sub>.

In this trial, the effect of using vitamin D<sub>3</sub> in the form of HYD on the production results in the period from weaning until slaughter was investigated. The dosage of total vitamin D<sub>3</sub> in the feed in this trial was above the Danish standard [1]. The feed was formulated to contain the same amount of total vitamin D<sub>3</sub> in the three groups:

Group 1:	Vitamin D <sub>3</sub> source - 100% vitamin D <sub>3</sub>
Group 2:	Vitamin D <sub>3</sub> source - 50% vitamin D <sub>3</sub> & 50% HYD
Group 3:	Vitamin D <sub>3</sub> source - 100% HYD

Overall, the trial revealed no differences in production value or health for neither weaners nor finishers regardless of the vitamin D<sub>3</sub> source added.

The highest content of total vitamin D<sub>3</sub> in the blood was found among the pigs given 50% vitamin D<sub>3</sub> and 50% HYD and that were actually given the highest dosage of total vitamin D<sub>3</sub>. The pigs given 100% HYD were actually given the lowest dosage of vitamin D<sub>3</sub> when the analysed content was converted into content of total vitamin D<sub>3</sub>.

The highest bone strength was found among the pigs given 100% vitamin D<sub>3</sub>. The differences in bone strength between the three groups were so small that they did not influence the well-being of the pigs.

### Background

Vitamin D<sub>3</sub> is one of the fat-soluble vitamins that are important to the turnover and deposit of calcium and phosphorus in bone tissue. Today, synthetic vitamin D<sub>3</sub> is added to pig feed in the form of cholecalciferol (D<sub>3</sub> cholecalciferol). For the pig to be able to utilize this form of vitamin D<sub>3</sub>, it must be transformed into 25-hydroxy vitamin D<sub>3</sub> (HYD) via the liver. This transformation from vitamin D<sub>3</sub> to HYD is necessary for vitamin D<sub>3</sub> to be transported to the tissue of the body. The addition of HYD to the feed ensures a supply of vitamin D<sub>3</sub> in a form that can be directly absorbed and deposited in the pig. The aim of using HYD was to ensure the pig a stable and adequate supply of vitamin D<sub>3</sub>. The standard for vitamin D<sub>3</sub> in pig feed is stated in international units (i.u.) per FUGp, but can also be converted into microgram: 40 i.u. vitamin D<sub>3</sub> equals 1 µg vitamin D<sub>3</sub>. In Denmark, the allowed maximum addition per kg complete pig diet is 2000 i.u. (=50 µg) total vitamin D<sub>3</sub>. The sum of these two vitamin D<sub>3</sub> sources is called total vitamin D<sub>3</sub>.

The trial included pigs from weaning at approx. five weeks of age until slaughter at approx. 100 kg. The effect of using increasing dosages of HYD as a replacement for the traditional vitamin D<sub>3</sub> source (cholecalciferol) was investigated on the production results. The feed was formulated to contain the same amount of total vitamin D<sub>3</sub> in the three groups. The dosage of total vitamin D<sub>3</sub> in the feed in this trial was above the Danish standard [1].

The aim was to investigate whether the productivity of the pigs was affected when the vitamin D<sub>3</sub> source was changed from vitamin D<sub>3</sub> to HYD. Health and mortality were recorded as secondary parameters. Bone

strength in the forelegs was analysed from random samples by way of DEXA scanning, and the total vitamin D<sub>3</sub> content in blood plasma was analysed in the same pigs.

### Materials and method

The trial was conducted in the period from July 2004 to December 2005 at Møllegården I/S, Holtevej 145, Serritslev, 9700 Brønderslev, which has health status blue SPF, with MS and PRRS. Approx. 1,000 weaners are produced every other week. The trial was conducted in the WTF (Wean to finish) facility. Each section contained eight pens with partially slatted floors and cover. Each pen had one tube feeder with two nipple drinkers in each pen partition and one extra drinking bowl.

The pigs were transferred to the trial over four rounds. Each round lasted approx. 18 weeks from weaning to slaughter. In each round, pigs were transferred over three times with a 14-day interval, and each round consisted of six replicates. The location of the individual treatments within a section was randomised.

The trial period included pigs from weaning at an age of approx. five weeks until slaughter at approx. 100 kg live weight. The weaner period is defined as the first six weeks post-weaning and the finisher period is the period from approx. 30 kg until slaughter at approx. 100 kg. Table 1 shows the groups in the trial and table 2 shows the form and amount of vitamin D<sub>3</sub> added to the diets.

### Groups

The groups are shown in table 1.

Group	1	2	3
Colour	Green	Red	Blue
<b>Diet 1 (first 14 days post-weaning)</b>			
Diet: FUT 1 Fi UK 123 FUgp/kg	100% vitamin D <sub>3</sub>	50% HYD + 50% vitamin D <sub>3</sub>	100% HYD
<b>Diet 2 (10-30 kg)</b>			
Diet: Profil 11 UK 113 FUgp/kg	100% vitamin D <sub>3</sub>	50% HYD + 50% vitamin D <sub>3</sub>	100% HYD
<b>Finisher feed (30-100 kg)</b>			
Diet: Profil 25 UK 105 FUgp/kg	100% vitamin D <sub>3</sub>	50% HYD + 50% vitamin D <sub>3</sub>	100% HYD

Group	1		2		3	
Colour	Green		Red		Blue	
	100% vitamin D <sub>3</sub>		50% HYD + 50% vitamin D <sub>3</sub>		100% HYD	
Weight interval, kg	9-30 kg	30-100 kg	9-30 kg	30-100 kg	9-30 kg	30-100 kg
Vitamin D <sub>3</sub> i.u./kg feed	2000	1200	1000	600	-	-
HYD, µg/kg feed	-	-	25	15	50	30

### Blocks

Each group included an average of 67 weaners in 24 double pens totalling 1617 pigs per treatment in the weaner period (weaning to 30 kg). At the beginning of the finisher period (at approx. 30 kg), half the pigs in each pen were sold thereby reducing the average number of pigs/double pen to 45, totalling 1075 pigs/treatment in the finisher period. Twenty-four pens with pigs on the same diet constituted a group. Three pens with pigs on the three diets constituted a block. The trial thus included three groups (treatments) and 24 blocks (replicates).

A computer-controlled system distributed the feed to each pen. The amount fed was calculated per pen and for each of the following periods:

1. Weaning until two weeks post-weaning
2. Two weeks post-weaning until approx. 30 kg (six weeks later)
3. Approx. 30 kg until slaughter at approx. 100 kg

The pigs' weight per pen was recorded at the same points in time. All pigs were weighed together on a weigh bridge. At 30 kg, the pigs that were to be sold were weighed first, and subsequently, the pigs that

stayed in the trial were weighed. Upon slaughter, the slaughter weight of the pigs was recorded at the slaughterhouse and the live weight was calculated on the basis of the formula "slaughter factor 1.31 \* carcass weight" [2].

### **Feed**

The same diets were used in all three groups, the only difference being the addition of the premixes, which all contained the same vitamins and minerals, except for the vitamin D<sub>3</sub> source. The premixes were delivered by DSM. The diets were all produced by DLG. The first two weeks post-weaning (9.4-12.7 kg) FUT UK was used; Profil 11 UK was used the following four weeks (12.7-32.5 kg); and Profil 25 UK from DLG was used in the finisher period (32.5-105 kg). The composition of the feed is shown in appendix 1. Pooled samples of the diets were analysed for content of energy (FUgp), lysine, methionine, cystine, threonine, calcium and phosphorus, and vitamin D<sub>3</sub> and HYD.

The standards for total vitamin D<sub>3</sub> in pig feed are [1]:

Weaners, 6-9 kg: 800 i.u. (20 µg) per FUgp

Weaners, 9-30 kg: 500 i.u. (12.5 µg) per FUgp

Finishers, 30-100 kg: 400 i.u. (10 µg) per FUgp

The allowed maximum dosage of total vitamin D<sub>3</sub> in pig feed in Denmark is 2000 i.u. (50 µg) per kg roughage, which was the dosage in the weaner diet in this trial. The finishers were given 1200 i.u. (30 µg) per kg feed, which was three times as much as prescribed by the standard.

### **Bone strength and blood analyses**

In the last part of the trial, 100 pigs were selected per group for determination of bone strength and vitamin D<sub>3</sub> content in the blood measured as 25 OH vitamin D<sub>3</sub> in blood plasma. The day before slaughter, the pigs were marked individually, and blood samples taken. The blood samples were centrifuged and plasma samples were forwarded to the DSM laboratory in Switzerland for analysis of vitamin D<sub>3</sub> content. The samples were blinded.

The individually marked animals were slaughtered, and their forelegs cut off in the cold room through the forearm. The forelegs were then packed, marked and frozen at -18°. They were forwarded to DEXA scanning at the Clinical Institute of the Royal Veterinary and Agricultural University. See [3] for a closer description of the DEXA equipment and calibration of the equipment.

Before scanning, the bones were thawed at room temperature for approx. 24 hours (figure 1). Both foretoes were scanned at the same time. A double determination was made. Both foretoes were scanned on the flat side (dorso-palmar projection) (figure 2). It was decided to scan the area called DEXA total (figure 3) of the foretoes. This area was selected because previous investigations have demonstrated that this is representative of the bone strength. The previous results based on sows showed a high correlation between DEXA total and DEXA spon, which is why it is only necessary to scan one of these areas in DEXA scannings. A linear correlation with the found DEXA total value BMD (Bone Mineral Density) [3] was previously found on sows for bone strength and content of ashes, phosphorus and calcium. It has not been established whether this correlation applies to finishers, which was not the aim of this trial. This trial attempted to document whether there was a difference in bone strength between the three groups of pigs given diets with varying dosages of vitamin D<sub>3</sub> and HYD.



Figure 1: Pig toes ready for scanning



Figure 2: Scanner for DEXA scanning of bones



Figure 3: X-ray of bones - bone strength is measured on the marked area (DEXA total).

### Liver and cuts

Liver, loin and streaky bacon were collected from 24 of the individually marked pigs, eight pigs from each group. Blood plasma samples, liver and cuts were forwarded to the National Food Institute, DTU, for analysis of vitamin D<sub>3</sub> metabolites. These analysis results are published separately [5].

### Statistics

The production value was calculated separately for each of the two production periods (weaner period and finisher period). Daily gain and feed conversion, and for the finishers also lean meat percentage, for each block were used for calculating the production value (PV) per place unit per year. The calculation of the feed conversion included the analysed content of FUGp for each diet. The production value was tested as the primary parameter. Mortality and treatments for diarrhoea were included as secondary parameters.

In the weaner period, the production value was calculated as  $(\text{kg gain} * \text{DKK per kg gain}) \div (\text{number of analysed FUGp} * \text{DKK per FUGp})$ . In the finisher period, the production value was calculated per place unit/year as  $\text{PV per pig} * (365 \text{ days/productive days per pig}) * \text{utilization of housing facility}$ . PV per pig was calculated as  $\text{sales price} \div \text{purchase price} \div \text{feed costs} \div \text{various costs}$ .

The value of gain (DKK 5.73 per kg gain) was calculated partly on the basis of the average start and finish weight for the entire trial and partly on the basis of the average price of the last five years (September 1, 2001 – September 1, 2006):

Pigs weighing 7 kg:	DKK 200 per pig, +/- DKK 8.50 per kg
Pigs weighing 30 kg:	DKK 340 per pig, + DKK 5.06 / ÷ DKK 4.90 per kg
Finishers:	DKK 8.68/kg, including bonus payment

The feed prices are the average of five years (September 1, 2001 – September 1, 2006):

Diet 1:	DKK 2.72 per FUGp
Diet 2:	DKK 1.47 per FUGp
Finisher feed:	DKK 1.18 per FUGp

Various costs are estimated at DKK 20/pig, and housing utilization at 95%.

The actual production value was not calculated, as the price of the product was not provided.

Data were subjected to an analysis of variance during which the primary parameter was the production value with weight at transfer as co-variable. The model included the following variables: round, block and group. Mortality and treatments for disease were secondary recordings in the trial and were also subjected to an analysis of variance with block and group as variables. Data were investigated for normal distribution and prevalence of outliers, and were subjected to an analysis of variance in SAS under the GLM procedure. Significant differences are stated at 5% level. To take the number of groups into consideration, a Bonferroni adjustment is made for two comparisons in pairs (groups 2 and 3 vs group 1). The result is shown as adjusted average for each group.

Bone strength and the blood's content total vitamin D<sub>3</sub> measured individually were also subjected to an analysis of variance in SAS under the GLM procedure. The treatment effect was tested within the various slaughter days. The correlation between the blood's content of total vitamin D<sub>3</sub> and bone strength was analysed together and for each of the treatments with a Pearson Correlation in SAS under the CORR procedure.

## **Results and discussion**

### **Feed analyses**

#### ***Nutrients (appendix 2)***

The FUT diet was used in the weight interval 9.4-12.7 kg. There was generally good agreement between the declared content and the analysed content. There was virtually no difference in nutrient content between the diets. The threonine content in the first delivery of the feed for group 2 was 19.4 mg per kg, approx. 2.5 times as high as in the diets for the other groups and the standard. In the second delivery, the threonine content was identical in all three groups. The deviation was probably caused by a mixing error during the production of the feed, but this does not seem to have affected the conclusion of the trial. The calcium content in diet 1 (9-13 kg) was 11% and 7%, respectively, below the standard in groups 2 and 3. A previous trial showed that a 25% calcium deficiency did not affect the productivity of weaners [4]. As the calcium content was only lower in the feed the first 14 days, it is not assumed to have affected the bone strength measured at slaughter. The pigs from which bones were taken for analysis were given the FUT diet from the second delivery in which the calcium standard was met.

Profil 11 was used in the weight interval 12.7-32.7 kg. There was fairly good agreement between the declared content and between the groups. Phosphorus in group 3 was lower than declared. The difference corresponded to the pigs in group 3 receiving 0.1 g/FUgp less than prescribed by the standard.

Profil 25 was used in the weight interval 32.7-105 kg (slaughter). There was good agreement between the declared content and the analysed content, and between the groups.

#### ***Vitamin D<sub>3</sub>***

The FUT diet was used in the weight interval 9.4-12.7 kg. The analysed content of vitamin D<sub>3</sub> was 12-13% lower than calculated in groups 1 and 2. The analyses revealed no vitamin D<sub>3</sub> content in the diet for group 3, as it was not added to this diet.

The content of HYD in the feed for groups 2 and 3 was 21% and 23% below the calculated content, respectively. HYD was not found in group 1, as it was not added to this diet.

Profil 11 was used in the weight interval 12.7-32.7 kg. The content of vitamin D<sub>3</sub> was 7% below the calculated content in group 1. In group 2, the content was 53% above the calculated content, and in group 3 an average content of 403 i.u./kg feed was found (corresponding to 10 µ per kg feed) – vitamin D<sub>3</sub> should not have been added in this group. One of the four deliveries did not contain vitamin D<sub>3</sub>, and the others ranged between 400 and 700 i.u./kg feed (corresponding to 10-18 µ per kg feed). As expected, the analyses revealed no content of HYD in the diet for group 1. Groups 2 and 3 had a HYD deficiency of 43% and 49%, respectively.

Profil 25 was used in the weight interval 32.7-105 kg (slaughter). In the feed for group 1, there was a vitamin D<sub>3</sub> deficiency of 28%. The content in the feed for group 2 corresponded with the calculated content, and, as expected, no vitamin D<sub>3</sub> was found in group 3. As expected, the analyses revealed no content of HYD in the diet for group 1. Groups 2 and 3 had a HYD deficiency of 26% and 37%, respectively.

The analysed content of total vitamin D<sub>3</sub> was 21% lower in group 3 compared with the control group. The content in group 2 and in the control group was largely identical (table 3).

Group	1			2			3		
	Control – 100% vitamin D <sub>3</sub>			50% HYD + 50% vitamin D <sub>3</sub>			100% HYD		
Weight, kg	9-13	13-33	33-105	9-13	13-33	33-105	9-13	13-33	33-105
Calculated D <sub>3</sub> in feed, i.u./kg/µg/kg	2000/50		1200/30	1000/25		600/15	-		-
Calculated HYD in feed, µg/kg	-	-	-	25		15	50		30
Analysed content converted to content of total vitamin D <sub>3</sub> , i.u./kg/µg/kg	1750/44	1867/47	1027/26	1625/41	2085/52	1046/26	1520/38	1442/36	809/20

### Vitamin D<sub>3</sub> in blood

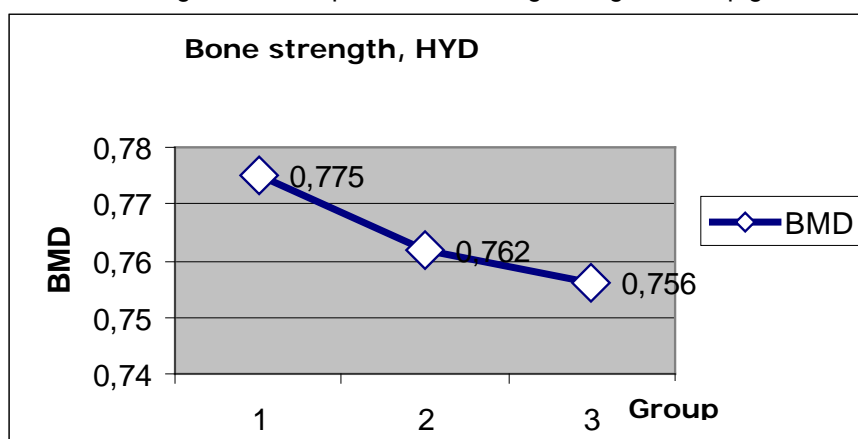
Analyses of the plasma samples demonstrated that there was no difference in the content of vitamin D<sub>3</sub> in the blood between groups 1 and 3. Compared with the other two groups, the pigs given 50% vitamin D<sub>3</sub> and 50% HYD had the highest content of vitamin D<sub>3</sub> in the blood (table 4). This corresponds with the fact that the content of total vitamin D<sub>3</sub> was highest in the end diet in group 2.

Group	1	2	3
	Control – 100% vitamin D <sub>3</sub>	50% HYD + 50% vitamin D <sub>3</sub>	100% HYD
Pigs	107	106	107
Average	22.6a	28.2b	20.8a
Spread (St dev)	6.8	7.3	6.3
Minimum	4.0	3.1	3.5
Maximum	46.7	41.6	46.1

a, b Values marked with different letters are significantly different

### Bone strength

DEXA scanning of the bones showed a significantly negative correlation between bone strength (BMD – bone mineral density) and the feed's calculated content of HYD. An increased content of HYD resulted in decreasing bone strength (figure 4). The highest bone strength was found in the pigs given 100% vitamin D<sub>3</sub>. The maximum difference in bone strength between the groups was 0.02. This difference is so small that it has no significance in practice to the leg strength of the pigs.

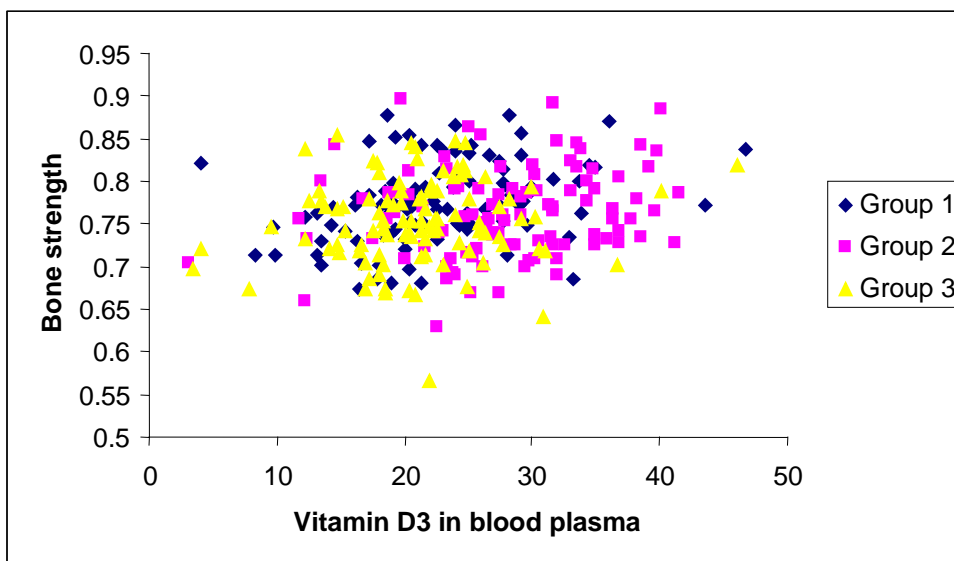


**Figure 4: Bone strength expressed via BMD (DEXA scanning) in relation to the content of vitamin D<sub>3</sub>/ HYD in the feed.**

The bone strength for finishers fed according to the standard is not known. It cannot be determined on the basis of this trial whether HYD has a greater effect when dosing total vitamin D<sub>3</sub> at the same level as the standard.

There was a significant but limited correlation of 0.21 between the blood's content of vitamin D<sub>3</sub> at slaughter and the bone strength. Figure 5 illustrates the correlation between the blood's content of vitamin D<sub>3</sub> and the bone strength. If you look at the correlation between the blood's content of vitamin D<sub>3</sub> and the

bone strength within the three groups, you will find a significant but limited correlation for groups 1 and 2 of 0.33 and 0.20, respectively.



**Figure 5: Bone strength in relation to analysed content of total vitamin D<sub>3</sub> in blood plasma (ng 25OH vitamin D<sub>3</sub> per ml plasma)**

### Health

There were no differences in mortality or the number of treatments regardless of vitamin D<sub>3</sub> source. In the weaner period, the pigs were treated for diarrhoea for 6.6 days on average. Mortality in that period averaged 0.5%. In the finisher period, each pig was treated on average 4.9 days, of which 4.7 were treatments for diarrhoea. Mortality in this period averaged 1.6%.

### Productivity and production value

There was no difference in production value between the groups in the weaner period (table 5).

<b>Table 5. Productivity, weaners</b>			
Groups	1	2	3
Pigs transferred	1617	1617	1617
Blocks	24	24	24
<b>Weaners 9.4-12.7 kg</b>			
Daily gain, g/day	232	220	231
Feed intake FUgp/day	0.39	0.38	0,40
Feed conversion, FUgp/kg	1.67	1.75	1.75
<b>Weaners 12.7 – 32.5 kg</b>			
Daily gain, g/day	585	592	599
Feed intake, FUgp/day	1.10	1.13	1.15
Feed conversion, FUgp/kg	1.90	1.92	1.93
<b>Weaners 9.4-32.5 kg</b>			
Daily gain, g/day	480	482	490
Feed intake, FUgp/day	0.89	0.91	0.93
Feed conversion, FUgp/kg	1.86	1.89	1.90
<b>Production value</b>			
DKK/pig <sup>1</sup>	61.8	61.3	61.7
Index	100	99	100



1) There must be a minimum difference of DKK 4.8 per pig or 7 index points between the groups in order for the difference to be significant.

There was no difference in production value between the groups in the finisher period (table 6).

Group	1	2	3
Pigs transferred	1073	1076	1075
Blocks	24	24	24
Finishers, 32.5-105.1 kg			
Daily gain, g/day	911	900	906
Feed intake, FUgp/day	2.34	2.33	2.36
Feed conversion, FUgp/kg	2.58	2.60	2.61
Lean meat %	61.2	61.3	61.4
Production value <sup>1</sup>			
DKK/place unit	533	518	518
Index	100	97	97

1) There must be a minimum difference of DKK 39.2 per pig or 7 index points between the groups in order for the difference to be significant.

## Conclusion

Overall for this trial, it can be concluded that there were no differences in production value or health for neither weaners nor finishers regardless of vitamin D<sub>3</sub> source.

The pigs given 50% HYD and 50% vitamin D<sub>3</sub> had the highest content of vitamin D<sub>3</sub> in the blood compared with the control group and the group given 100% HYD. This is probably caused by the fact that the pigs given 100% HYD were actually given the lowest dose of vitamin D<sub>3</sub>.

The highest bone strength was found in the pigs given 100% vitamin D<sub>3</sub> despite the fact that they were not given the highest amount of total vitamin D<sub>3</sub> in the feed. The differences in bone strength between the three groups were so small that they did not influence the well-being of the pigs.

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**Participants**

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**Trial:** 828

**Ingredient composition in the diets for groups 1-3, %. The only variation was the vitamin D<sub>3</sub> content of the premix**

Ingredients	FUT	Profil 11	Profil 25
Barley	50.00	28.00	22.30
Wheat	19.07	39.68	36.50
Soybean meal	-	18.00	19.90
Wheat bran	-	-	10.00
Fishmeal	9.04	5.00	-
Dried whey	6.00		-
Oats	5.00		5.00
Palm oil	3.16 <sup>1</sup>	2.90	1.30
Potato protein concentrate	3.00	-	-
Molasses	-	2.00	2.00
Monocalcium phosphate	1.10	0.86	0.27
Feed lime	-	0.94	1.46
Bio lysine	1.08		0.24
Calcium formate	0.68	0.80	-
Acid one Dry	0.60	-	-
Premix	0.40	0.40	0.20
Lysine, liquid conc.	-	0.57	-
Methionine	0.28	0.07	-
Dietary salt	0.22	0.39	0.61
Threonine	0.22	0.25	0.09
Vitamin E 25,000	0.12	-	0.05
Phytase	0.03	0.03	0.03
Porzyme 8300/9310	-	0.10	0.05
Aromatic compounds	-	0.01	-

## Nutrient content, diet 1: FUT

Nutrient	Declared	Analysed <sup>1</sup>		
		1	2	3
Group				
Crude protein, g/kg	17.1	18.2	17.9	17.7
Crude fat, g/kg	6.4	5.9	5.8	5.9
FUgp per 100 kg	-	125	126	126
Lysine, g/kg	14.3	14.4	13.6	13.7
Methionine, g/kg	-	6.0	6.0	6.0
Cystine, g/kg		2.7	2.6	2.7
Threonine, g/kg	-	9.6	14.9 <sup>2</sup>	8.7
Calcium, g/kg <sup>3</sup>	-	8.5	7.8	8.2
Phosphorus, g/kg <sup>3</sup>	6.5	6.8	6.7	7.0
D <sub>3</sub> cholecalciferol group 1 <sup>3</sup> i.u. per kg (µg/kg)	2000 (50)	1750 (44)	-	-
D <sub>3</sub> cholecalciferol group 2 <sup>3</sup> i.u. per kg (µg/kg)	1000 (25)	-	825 (21)	-
D <sub>3</sub> cholecalciferol group 3 <sup>3</sup> i.u. per kg (µg/kg)	0	-	-	0
HyD group 1 <sup>3</sup> µg per kg	0	0	-	-
HyD group 2 <sup>3</sup> µg per kg	25	-	20	-
HyD group 3 <sup>3</sup> µg per kg	50	-	-	38

1. Average of seven analyses.

2. In the first delivery, 19.4 g/kg was found; the second delivery corresponded to the content in groups 1 and 3.

3. Average of two analyses.

## Nutrient content, diet 2: Profil 11, DLG

Nutrient	Declared (av. 5 deliveries)	Analysed <sup>1</sup>		
		1	2	3
Group				
Crude protein, g/kg	18.4	18.9	18.6	18.4
Crude fat, g/kg	5.2	4.9	4.8	5.0
FUgp per 100 kg	113	112	112	112
Lysine, g/kg	12.4	12.8	13.0	12.2
Methionine, g/kg	3.8	3.6	3.8	3.6
Cystine, g/kg		3.3	3.2	3.3
Threonine, g/kg	-	7.8	7.9	7.5
Calcium, g/kg <sup>2</sup>	9.2	10.8	10.6	9.9
Phosphorus, g/kg <sup>2</sup>	6.1	6.3	6.4	5.9
D <sub>3</sub> cholecalciferol group 1 <sup>2</sup> i.u. per kg	2000	1867	-	-
D <sub>3</sub> cholecalciferol group 2 <sup>2</sup> i.u. per kg	1000	-	1525	-
D <sub>3</sub> cholecalciferol group 3 <sup>2</sup> i.u. per kg	0	-	-	402
HyD group 1 <sup>3</sup> µg per kg	0	0	-	-
HyD group 2 <sup>3</sup> µg per kg	25	-	14	-
HyD group 3 <sup>3</sup> µg per kg	50	-	-	26

1. Average of nine analyses.

2. Average of three analyses.

3. Average of five analyses.

## Nutrient content, diet 2: Profil 25, DLG

Nutrient Group	Declared	Analysed <sup>1</sup>		
		1	2	3
Crude protein, g/kg	16.1	16.5	16.4	16.3
Crude fat, g/kg	3.9	3.8	3.7	3.7
FUgp per 100 kg	105	104	104	104
Lysine, g/kg	9.0	9.4	9.3	9.3
Methionine, g/kg	2.6	2.5	2.4	2.4
Cystine, g/kg	-	3.2	3.2	3.1
Threonine, g/kg	-	6.3	6.3	6.2
Calcium, g/kg <sup>2</sup>	7.1	7.1	7.4	7.5
Phosphorus, g/kg <sup>2</sup>	4.7	4.9	5.2	5.1
D <sub>3</sub> cholecalciferol group 1 <sup>3</sup> i.u. per kg	1200	987	-	-
D <sub>3</sub> cholecalciferol group 2 <sup>3</sup> i.u. per kg	600	-	606	49
D <sub>3</sub> cholecalciferol group 3 <sup>3</sup> i.u. per kg	0	-	-	48.8 <sup>4</sup>
HyD group 1 <sup>3</sup> µg per kg	0	1	-	-
HyD group 2 <sup>3</sup> µg per kg	15	-	11	-
HyD group 3 <sup>3</sup> µg per kg	30	-	-	19

1. Average of six analyses.
2. Average of two analyses.
3. Average of eight analyses.
4. One sample with 390 i.u. per kg.