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(75) Inventors/Applicants (for US only): ELLIOTT, Robert [AU/AU]; 25 Primley St., Pullenvale, Brisbane, QLD 4069 (AU). WEBER, Gilbert [CH/CH]; Im Hofacker 5, CH-4312 Magden (CH).


(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CE, CG, CI, CM, GA, GN, GQ, Gw, ML, MR, NE, SN, TD, TG).

(54) Title: FOOD SUPPLEMENTATION COMPOSITION CONTAINING ONE OR MORE VITAMIN D₃ COMPOUNDS AND ONE OR MORE MAGNESIUM SALTS

(57) Abstract: Use of one or more vitamin D₃ compounds chosen from the group 1α,25-dihydroxy vitamin D₃, 25-hydroxy vitamin D₃, 1α,25-dihydroxy vitamin D₃, in combination with one or more magnesium salts to prevent milk fever in animals.
Food supplementation composition containing one or more vitamin D₃ compounds and one or more magnesium salts

The present invention relates to a food supplementation composition containing one or more vitamin D₃ compounds chosen from the group 1-α-hydroxy vitamin D₃, 25-hydroxy vitamin D₃, 1-α-, 25-dihydroxy vitamin D₃, in combination with one or more magnesium salts. More particularly, the invention relates to a food supplementation composition containing the above mentioned ingredients for the prevention of milk fever in animals, particularly in dairy cows.

The invention further relates to the use of one or more vitamin D₃ compounds chosen from the group 1-α-hydroxy vitamin D₃, 25-hydroxy vitamin D₃, 1-α-, 25-dihydroxy vitamin D₃, in combination with one or more magnesium salts, especially magnesium chloride and/or magnesium sulphate, in the manufacture of a food or veterinary composition for the prevention of milk fever in animals, particularly dairy cows.

In another aspect, the invention relates to a method for the prevention of milk fever in animals, particularly dairy cows, which comprises administering to an animal in need of such treatment one or more vitamin D₃ compounds chosen from the group 1-α-hydroxy vitamin D₃, 25-hydroxy vitamin D₃, 1-α-, 25-dihydroxy vitamin D₃, in combination with one or more magnesium salts, especially magnesium chloride and/or magnesium sulphate.

The transition between late pregnancy and early lactation (3-4 weeks post partum) is a high risk period for many metabolic disorders in dairy cows. In particular the failure to maintain blood calcium concentrations after calving due to the rapid demand for colostrum production can lead to both hypocalcaemia and clinical milk fever.

Dairy cows are normally fed on predominantly forage based rations before calving and the resulting high potassium intakes can result in metabolic alkalosis. In order to prepare the cow for calving, anionic supplements such as magnesium chloride or magnesium sulphate are normally included as part of a pre-calving supplementation programme.

In accordance with the present invention it has now been found that the above mentioned problems can be eliminated or substantially ameliorated by administering to the animals an
effective amount of one or more vitamin D₃ compounds chosen from the group 1-α-hydroxy vitamin D₃, 25-hydroxy vitamin D₃, 1-α-,25-dihydroxy vitamin D₃, in combination with one or more magnesium salts, especially magnesium chloride and/or magnesium sulphate.

A food supplementation composition containing one or more vitamin D₃ compounds chosen from the group 1-α-hydroxy vitamin D₃, 25-hydroxy vitamin D₃, 1-α-, 25-dihydroxy vitamin D₃, in combination with one or more magnesium salts is therefore one preferred embodiment of the present invention.

According to the present invention one or more vitamin D₃ compounds and one or more magnesium salts are suitably administered together with the food. Food may be supplemented by admixing one or more of the vitamin D₃ compounds according to the present invention – e.g. 25-hydroxy vitamin D₃ as a commercial formulation such as available under the Trademark ROVIMIX® Hy-D® 1.25 % – and one or more magnesium salts to regular food. The terms “food” or “food composition” as used herein comprise solid and liquid food as well as drinking fluids such as drinking water.

A dry food composition for dairy cows according to the present invention contains, if it is used as the sole vitamin D₃ compound, 25-hydroxy vitamin D₃ preferably in an amount of 100 μg per kg of the dry food composition to 1000 μg per kg of the dry food composition, more preferably in an amount of 250 μg per kg of the dry food composition to 750 μg per kg of the dry food composition, most preferred about 500 μg per kg of the dry food composition.

As 1-α-hydroxy vitamin D₃ and 1-α-, 25-dihydroxy vitamin D₃ are both approximately 10 times more active than 25-hydroxy vitamin D₃, their amount in a dry food composition should be adapted accordingly. The same applies for mixtures of two or three of the vitamin D₃ compounds according to the present invention. It is most preferred to use 25-hydroxy vitamin D₃ as the sole vitamin D₃ compound.

The food composition further comprises from 5 g per kg of the dry food composition to 15 g per kg of the dry food composition of one or more magnesium salts, especially magnesium chloride and/or magnesium sulphate. Magnesium chloride is most preferred.

According to the present invention it is further advantageous if the composition also contains one or more of the following ingredients: Vitamin A, Vitamin E, Biotin, copper (e.g. as CuSO₄), zinc (e.g. as ZnSO₄), cobalt (e.g. as CoSO₄), selenium (e.g. as Na₂SeO₃), iodine (e.g. as KI), manganese (e.g. as MnSO₄) and/or calcium (e.g. as CaSO₄).
It is preferred to use calcium sulphate as calcium carrier in a composition according to the present invention.

In a further preferred embodiment of the present invention the composition is a premix, i.e. one or more vitamin D₃ compounds according to the present invention together with one or more magnesium salts are – e.g. as a formulated powder – added to other minerals, vitamins, amino acids and/or trace elements in a higher concentration in order to form the premix. For use the premix is added to and thoroughly mixed with a regular animal food to achieve even distribution therein.

A premix according to the present invention may be prepared by adding the active ingredients to regular food components in a concentration of from about 5 mg per kg of the premix to about 50 mg per kg of the premix of 25-hydroxy vitamin D₃ – if 25-hydroxy vitamin D₃ is the sole vitamin D₃ compound – and from about 250 g per kg of the premix to about 750 g per kg of the premix of one or more magnesium salts, especially magnesium chloride and/or magnesium sulphate. If 2 kg of such premix are added per 100 kg of regular food this typically meets the individual need of the animal by normal food consumption.

When the composition is prepared in the form of a premix the premix preferably comprises from 3 to 6 mg 25-hydroxy vitamin D₃ (if 25-hydroxy vitamin D₃ is the sole vitamin D₃ compound), and further from 80,000 to 120,000 IU Vitamin A, from 1000 to 3000 IU Vitamin E, from 10 to 20 mg Biotin, from 200 to 300 mg copper (as CuSO₄), from 300 to 600 mg zinc (as ZnSO₄), from 5 to 10 mg cobalt (as CoSO₄), from 1 to 6 mg selenium (as Na₂SeO₃), from 5 to 10 mg iodine (as KI) and/or from 200 to 400 mg manganese (as MnSO₄) in addition to the magnesium chloride or sulphate.

For mature dairy cows preferable daily dosages of 25-hydroxy vitamin D₃ per cow – if 25-hydroxy vitamin D₃ is used as the sole vitamin D₃ compound – are in the range of from 1 to 10 mg, preferably from 1 to 6 mg, most preferred about 3 mg. If 1-α-hydroxy vitamin D₃ and/or 1-α-25, dihydroxy vitamin D₃ or mixtures of vitamin D₃ compounds containing one or both of these are used, the individual amount of each vitamin D₃ compound should be adapted accordingly.

Preferable daily dosages of one or more magnesium salts – preferably magnesium chloride and/or magnesium sulphate, most preferred magnesium chloride – per cow are in the range of from 50 to 150 g, preferably from 100 to 150 g, most preferred about 150 g.
According to the present invention it is preferred to start administering one or more vitamin D₃ compounds chosen from the group 1-α-hydroxy vitamin D₃, 25-hydroxy vitamin D₃, 1-α-, 25-dihydroxy vitamin D₃, in combination with one or more magnesium salts, especially magnesium chloride and/or magnesium sulphate, most preferred magnesium chloride – one to four weeks prior to calving, most preferred approximately two weeks prior to calving.

The invention is further illustrated by the following examples.
Examples:

Example 1

Four mature rumen fistulated cattle (approximately 600 kg liveweight) were held in individual pens and offered 12 kg/day of good quality lucerne chaff throughout the experiment. After a 2 week adaptation to this diet the following treatments were applied daily over 2 weeks to each animal in a Latin Square Design:

1. no supplement (control)
2. MgCl₂ (150 mg magnesium chloride),
3. ROVIMIX® Hy-D® 1.25 % (5 mg 25-hydroxy vitamin D₃)
4. MgCl₂ plus ROVIMIX® Hy-D® 1.25 %

Each treatment was dosed into the rumen through a cannula each morning during the 2 week experimental period. Blood samples were collected on days 7, 13 and 14 and urine samples on days 12, 13 and 14 of the period. Urine pH was measured at time of collection; blood plasma was decanted after centrifugation and analyzed for calcium, magnesium, potassium, sodium and phosphorus by inductively coupled plasma atomic emission spectrophotometry. The results were analysed as a latin square design making comparisons between treatments after removal of any animal or period effects. The lucerne chaff was assayed for macro cations together with chloride and sulphur concentrations.

The results are shown in Table 1.

The combination of magnesium chloride plus ROVIMIX® Hy-D® 1.25 % resulted in an amplification in calcium and phosphorus mobilisation from bone mass that exceeds the enhancement observed when using magnesium chloride alone.
### Table 1

**Effects of MgCl₂ and Hy-D on urine pH, and urine and blood plasma minerals**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Liveweight (kg)</th>
<th>Feed Intake (kg/day)</th>
<th>Urine pH</th>
<th>Urine Composition (mg/L)</th>
<th>Blood Plasma Composition (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ca²⁺</td>
<td>Mg²⁺</td>
</tr>
<tr>
<td>Control</td>
<td>616</td>
<td>11.8</td>
<td>7.00a</td>
<td>226a</td>
<td>337</td>
</tr>
<tr>
<td>Hy-D⁰</td>
<td>602</td>
<td>11.8</td>
<td>7.17a</td>
<td>224a</td>
<td>264</td>
</tr>
<tr>
<td>Mg</td>
<td>602</td>
<td>11.8</td>
<td>6.20b</td>
<td>397b</td>
<td>411</td>
</tr>
<tr>
<td>Mg + Hy-D⁰</td>
<td>598</td>
<td>11.2</td>
<td>5.82b</td>
<td>592c</td>
<td>323</td>
</tr>
</tbody>
</table>

**Significance**

- NS
- P < 0.05

**F Value**

- 1.24
- 2.28
- 4.86
- 8.64
- 1.5
- 1.25
- 2.41
- 0.66
- 0.75
- 2.03
- 1.31
- 0.91

4.76 = 0.05
Example 2

A feed formulation for dry cows before calving containing 25-hydroxy vitamin D₃ and magnesium chloride can be prepared as follows (Dry Matter basis):

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>% by weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize silage</td>
<td>30</td>
</tr>
<tr>
<td>Grass silage</td>
<td>20</td>
</tr>
<tr>
<td>Cereal grain</td>
<td>25</td>
</tr>
<tr>
<td>Long hay</td>
<td>15</td>
</tr>
<tr>
<td>Protein meal</td>
<td>8</td>
</tr>
<tr>
<td>Vitamins and trace elements premix¹</td>
<td>2</td>
</tr>
</tbody>
</table>

¹ containing the following per 100 g of premix:
- 3 mg ROVIMIX® Hy•D® 1.25 %
- 100,000 IU Vitamin A,
- 2000 IU Vitamin E,
- 15 mg Biotin,
- 250 mg copper (as CuSO₄)
- 450 mg zinc (as ZnSO₄)
- 7.5 mg cobalt (as CoSO₄)
- 3 mg selenium (as Na₂SeO₃)
- 7.5 mg iodine (as KI),
- 300 mg manganese (as MnSO₄)
- 20 g magnesium (as MgCl₂)

The ingredients are mixed together.

Example 3

A premix for a dairy cow food containing 25-hydroxy vitamin D₃ and magnesium chloride can be prepared as follows (active content in brackets):

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>% by weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>ROVIMIX® Hy•D® 1.25 %</td>
<td>0.2</td>
</tr>
<tr>
<td>Vitamin A 1000</td>
<td>0.05</td>
</tr>
<tr>
<td>Vitamin E (50 %)</td>
<td>2</td>
</tr>
<tr>
<td>Biotin (2 %)</td>
<td>0.375</td>
</tr>
<tr>
<td>CuSO₄ (25 %)</td>
<td>0.5</td>
</tr>
<tr>
<td>ZnSO₄ (80 %)</td>
<td>0.281</td>
</tr>
<tr>
<td>Ingredient</td>
<td>Amount</td>
</tr>
<tr>
<td>--------------------</td>
<td>---------</td>
</tr>
<tr>
<td>CoSO₄ (20 %)</td>
<td>0.0185</td>
</tr>
<tr>
<td>Na₂SeO₃ (44 %)</td>
<td>0.0035</td>
</tr>
<tr>
<td>KI (68 %)</td>
<td>0.0055</td>
</tr>
<tr>
<td>MnSO₄ (31 %)</td>
<td>0.484</td>
</tr>
<tr>
<td>CaSO₄</td>
<td>21.08</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>75</td>
</tr>
</tbody>
</table>

All ingredients are carefully mixed together and 2 % (2 kg/100 kg of food) of this premix is added to the dry cow food.
Claims:

1. Use of one or more vitamin D₃ compounds chosen from the group 1-α-hydroxy vitamin D₃, 25-hydroxy vitamin D₃, 1-α-,25-dihydroxy vitamin D₃, in combination with one or more magnesium salts to prevent milk fever in animals.

2. Use of one or more vitamin D₃ compounds chosen from the group 1-α-hydroxy vitamin D₃, 25-hydroxy vitamin D₃, 1-α-,25-dihydroxy vitamin D₃, in combination with one or more magnesium salts for the manufacture of a food composition to prevent milk fever in animals.

3. Use according to one of the claims 1 or 2, wherein 25-hydroxy vitamin D₃ is used as sole vitamin D₃ compound.

4. Use according to one of the claims 1 or 2, wherein the magnesium salt is magnesium chloride and/or magnesium sulphate.

5. Use according to claim 2, wherein the food is a dry food composition which comprises from about 100 µg per kg to about 1000 µg per kg of 25-hydroxy vitamin D₃.

6. Use according to any of the preceding claims, wherein the animal is a dairy cow.

7. Method for preventing milk fever in animals said method comprising the step of administering to an animal in need of such treatment an amount of 1 mg to 10 mg of 25-hydroxy vitamin D₃ and from 50 g to 150 g of magnesium chloride and/or magnesium sulphate per day.

8. Method according to claim 7, wherein the animal is a dairy cow.

9. Method according to one of the claims 7 or 8, wherein the administering starts one to four weeks prior to calving.

10. Food supplementation composition containing one or more vitamin D₃ compounds chosen from the group 1-α-hydroxy vitamin D₃, 25-hydroxy vitamin D₃, 1-α-, 25-dihydroxy vitamin D₃, in combination with one or more magnesium salts.
**INTERNATIONAL SEARCH REPORT**

**INTERNATIONAL APPLICATION NO**

PCT/EP2007/007996

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**A. CLASSIFICATION OF SUBJECT MATTER**

INV. A23K1/16  A23K1/175  A23K1/18  A61K31/06  A61K31/593

According to International Patent Classification (IPC) or to both national classification and IPC

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**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

A23K  A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, FSTA, EMBASE, BIOSIS, COMPENDEX

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**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

<table>
<thead>
<tr>
<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y</td>
<td>WO 2006/072252 A (HANSEN RICHARD BRINCH [DK]) 13 July 2006 (2006-07-13) page 12, line 8 - line 9; claims 1,12-15</td>
<td>1-10</td>
</tr>
<tr>
<td>Y</td>
<td>US 6 322 821 B1 (REGISTER JACK W [US]) 27 November 2001 (2001-11-27) column 6, line 8 - line 11; tables 3,5 column 7, line 18 - line 21 column 5, line 2 - line 6</td>
<td>1-10</td>
</tr>
<tr>
<td>Y</td>
<td>US 3 646 203 A (LUCA HECTOR F DE) 29 February 1972 (1972-02-29) column 2, line 15 - line 22; claim 1</td>
<td>1-10</td>
</tr>
</tbody>
</table>

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**X** Further documents are listed in the continuation of Box C.  
**X** See patent family annex.

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* Special categories of cited documents:
  * "A" document defining the general state of the art which is not considered to be of particular relevance
  * "E" earlier document but published on or after the international filing date
  * "L" document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
  * "O" document referring to an oral disclosure, use, exhibition or other means
  * "P" document published prior to the international filing date but later than the priority date claimed

* "I" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

* "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

* "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is considered in combination with one or more other such documents, such combination being obvious to a person skilled in the art

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Date of the actual completion of the international search: 13 November 2007

Date of mailing of the international search report: 05/12/2007

Name and mailing address of the ISA:

European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 H Wassenaar Tel (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016

Authorized officer:

Smeets, Dieter

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Form PCT/ISA/210 (second sheet) (April 2005)

page 1 of 2
<table>
<thead>
<tr>
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<th>Relevant to claim No.</th>
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<tr>
<td>X</td>
<td>US 4 004 003 A (BABCOCK JOHN C ET AL) 18 January 1977 (1977-01-18) claims 2,3</td>
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<tr>
<td>X</td>
<td>EP 0 191 489 A (CHUGAI PHARMACEUTICAL CO LTD [JP]) 20 August 1986 (1986-08-20) example 6</td>
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</tbody>
</table>
Continuation of Box II.1

Although claims 1, 3(part), 4(part), 6(part), 7-9 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the composition.

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Continuation of Box II.1

Claims Nos.: 1, 3(part), 4(part), 6(part), 7-9

Rule 39.1(iv) PCT – Method for treatment of the human or animal body by therapy
INTERNATIONAL SEARCH REPORT

Box No. II  Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☑ Claims Nos.: 1, 3(part), 4(part), 6(part), 7-9
   because they relate to subject matter not required to be searched by this Authority, namely:
   see FURTHER INFORMATION sheet PCT/ISA/210

2. ☐ Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.: because they are dependant claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III  Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☑ No required additional search fees were timely paid by the applicant. Consequently, this International search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant’s protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant’s protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (2)) (April 2005)
<table>
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<td></td>
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<td>EP 1838292 A1</td>
<td>03-10-2007</td>
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<td>US 6322821 B1</td>
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<td>EP 0191489 A</td>
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