

19



**Octrooi centrum
Nederland**

11

2028362

12 B1 OCTROOI

21 Aanvraagnummer: **2028362**

22 Aanvraag ingediend: **1 juni 2021**

51 Int. Cl.:

A61K 31/593 (2021.01) **A61K 33/06** (2021.01) **A61K 33/08** (2021.01) **A61K 33/10** (2021.01) **A61K 38/43** (2022.01) **A23K 20/174** (2022.01) **A23K 20/189** (2022.01) **A23K 20/24** (2022.01)

30 Voorrang:

-

41 Aanvraag ingeschreven:

14 december 2022

43 Aanvraag gepubliceerd:

-

47 Octrooi verleend:

14 december 2022

45 Octrooischrift uitgegeven:

15 december 2022

73 Octrooihouder(s):

Intracare BV te Veghel

72 Uitvinder(s):

Carly Vulders te Veghel

74 Gemachtigde:

dr. A. Ellens c.s. te Wageningen

54 **COMPOSITION FOR IMPROVING CALCIUM AND PHOSPHORUS REGULATION IN POULTRY AND USES THEREOF**

57 The present application relates to orally administrable compositions comprising vitamin D₃, a calcium source and a phytase suitable for improving the calcium and phosphorus regulation and for reinforcing the eggshell quality in poultry. More particularly, the compositions are added to the drinking water of the poultry. Also methods to improve the calcium and phosphorus regulation and eggshell quality in poultry by administering said composition are disclosed.

**COMPOSITION FOR IMPROVING CALCIUM AND PHOSPHORUS REGULATION
IN POULTRY AND USES THEREOF**

FIELD OF THE INVENTION

5 The present application relates generally to orally administrable compositions for improving the calcium and phosphorus regulation and for reinforcing the eggshell quality in poultry. Also methods to improve the calcium and phosphorus regulation and eggshell quality in poultry by administering said composition are disclosed.

10 **BACKGROUND OF THE INVENTION**

Optimal intestinal absorption of fundamental minerals and nutrients is essential to achieve full economic potential of modern layer poultry farming. Nutritional elements, such as vitamin D₃, calcium and phosphorus, play a crucial role in the musculoskeletal integrity of the animals as well as in the development process of the eggshell. A drop in quality of the eggshells, and in particular, of the mechanical resistance of the eggshells, has given rise to a substantial increase in the percentage of broken, cracked or microcracked eggs, giving rise to a substantial loss in the product during transportation. Moreover, the reduction in quality of the eggshells requires the breeder to reduce the productive life of the laying poultry because this decrease in quality shows up most particularly after the 60th week of the life of the laying poultry.

20 Among the various nutritional factors that are necessary for proper eggshell formation in poultry, calcium and phosphorus probably play the most important role, because both calcium phosphorus are highly involved in the formation of the eggshell. Furthermore, it is well established that also for laying hens vitamin D₃ is key to the maintenance of calcium homeostasis by stimulating its absorption and uptake.

25 Low availability of phosphorus in plant-based poultry diets has led to inorganic phosphorus supplementation of the poultry diets. Owing to phosphorus being one of the most expensive nutrients in poultry diets, increasing inorganic phosphorus in the diet, also increases feed cost. Furthermore, due to the public concern surrounding the contribution of phosphorus from animal excreta to environmental pollution, the industry is attempting to reduce phosphorus levels in commercial layer diets by reducing the margin of safety of this nutrient in feed formulation. Exogenous phytases have been used in poultry diets to liberate phosphorus and other minerals such as calcium, to reduce phosphorus pollution in poultry excreta and to reduce feed cost since they reduce the need for high

30

levels of phosphorus in the feed. Nevertheless, so far, research studies have been focused on broilers only, but very little work has been carried out on laying hens.

SUMMARY

5 Provided herein are compositions, more particularly orally administrable compositions for improving the calcium and phosphorus regulation and for reinforcing the eggshell quality in poultry. More particularly orally administrable compositions are provided comprising vitamin D₃, a calcium source and a phytase. Also provided are those compositions for use in treatment and/or prevention of a disturbed calcium and phosphorus regulation and for preventing a reduced quality of the eggshell in
10 poultry. The compositions are particularly suitable for improving the eggshell quality in poultry .

The applicants have found that compositions comprising vitamin D₃, a combination of an inorganic and organic calcium salt as calcium source and a stabilized phytase provide synergistic effects on the calcium and phosphorus regulation in poultry thereby improving the musculoskeletal integrity of the animals and improving the eggshell quality of poultry.

15 The present application thus provides an orally administrable composition comprising vitamin D₃ or a derivative or analogue thereof, a calcium source and a stabilized phytase.

In particular embodiments, the orally administrable composition comprises at least 200 IU/l; preferably at least 400 IU/l, vitamin D₃ or a derivative or analogue thereof.

In particular embodiments, the calcium source is an organic calcium salt, an inorganic calcium salt, or
20 a combination thereof, or any hydrates thereof. In particular embodiments, the calcium source is a combination of an organic calcium salt and an inorganic calcium salt, or any hydrates thereof.

In particular embodiments, the composition comprises a phytase that is selected from type A phytase or type B phytase. In some embodiments, the phytase is a fungal phytase, such as a phytase derived from the *Aspergillus niger* (*A. niger*) fungal strain. In some embodiments, the phytase is a bacterial
25 phytase, such as a phytase derived from *Escherichia coli* (*E. coli*).

The application also provides the orally administrable composition as disclosed herein for use in treatment and/or prevention of a disturbed calcium and phosphorus regulation in poultry. In some embodiments, the orally administrable composition as disclosed herein is provided for use in the prevention of a reduced eggshell quality of poultry.

30 In a further aspect, methods are provided for maintaining a normal calcium and phosphorus regulation in poultry wherein the orally administrable composition as disclosed herein is provided to the poultry.

In still another aspect, methods are provided for maintaining and/or improving the eggshell quality of poultry by administering the orally administrable composition as disclosed herein to the poultry.

In particular embodiments, the orally administrable composition as disclosed herein is administered via the drinking water to the poultry. Particularly, the composition is administered via the drinking
5 water to the poultry in a dilution of from 0.1 to 10% v/v; more preferably in a dilution of from 0.01% to 1% v/v.

In particular embodiments, the orally administrable composition as disclosed herein is provided to the poultry during at least 3 consecutive days; preferably during at least 3 consecutive days wherein the composition is provided continuously or ad libitum to the poultry. In some further embodiments, the
10 composition as disclosed herein is provided ad libitum to the poultry for 3 to 7 consecutive days per week.

In particular embodiments, the poultry is selected from layer poultry, parent stock or broilers. Even more preferred, the poultry is layer poultry.

In particular embodiments, the poultry is selected from chicken, ducks, or quails.

15

BRIEF DESCRIPTION OF DRAWINGS

Figure 1. Percentage of damaged eggs in a commercial layer farm after treatment with a composition comprising vitamin D₃, calcium chloride, calcium gluconate monohydrate and phytase. The composition was provided in the drinking water of layer hens from the age of 60 weeks. The arrow
20 indicates the start of administration of the composition comprising vitamin D₃, calcium chloride, calcium gluconate monohydrate and phytase. The straight line represents the trend of percentage of damaged eggs prior to the use of the composition.

Figure 2. Comparison of the damaged egg rates in hens treated with the composition comprising vitamin D₃, calcium chloride, calcium gluconate monohydrate and phytase, and a control group that
25 did not receive the composition. 60-week old animals were treated during 11 days with the composition or with a control solution and the % of damaged eggs was evaluated.

DESCRIPTION OF EMBODIMENTS

As used herein, the singular forms “a”, “an”, and “the” include both singular and plural referents
30 unless the context clearly dictates otherwise.

The terms "comprising", "comprises" and "comprised of" as used herein are synonymous with "including", "includes" or "containing", "contains", and are inclusive or open-ended and do not exclude additional, non-recited members, elements or method steps. The terms also encompass "consisting of" and "consisting essentially of", which enjoy well-established meanings in patent terminology.

The recitation of numerical ranges by endpoints includes all numbers and fractions subsumed within the respective ranges, as well as the recited endpoints.

The terms "about" or "approximately" as used herein when referring to a measurable value such as a parameter, an amount, a temporal duration, and the like, are meant to encompass variations of and from the specified value, such as variations of +/-10% or less, preferably +/-5% or less, more preferably +/-1% or less, and still more preferably +/-0.1% or less of and from the specified value, insofar such variations are appropriate to perform in the disclosed invention. It is to be understood that the value to which the modifier "about" refers is itself also specifically, and preferably, disclosed.

Whereas the terms "one or more" or "at least one", such as one or more members or at least one member of a group of members, is clear per se, by means of further exemplification, the term encompasses *inter alia* a reference to any one of said members, or to any two or more of said members, such as, e.g., any ≥ 3 , ≥ 4 , ≥ 5 , ≥ 6 or ≥ 7 etc. of said members, and up to all said members. In another example, "one or more" or "at least one" may refer to 1, 2, 3, 4, 5, 6, 7 or more.

The discussion of the background to the invention herein is included to explain the context of the invention. This is not to be taken as an admission that any of the material referred to was published, known, or part of the common general knowledge in any country as of the priority date of any of the claims.

Throughout this disclosure, various publications, patents and published patent specifications are referenced by an identifying citation. All documents cited in the present specification are hereby incorporated by reference in their entirety. In particular, the teachings or sections of such documents herein specifically referred to are incorporated by reference.

Unless otherwise defined, all terms used in disclosing the invention, including technical and scientific terms, have the meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. By means of further guidance, term definitions are included to better appreciate the teaching of the invention. When specific terms are defined in connection with a particular aspect of the invention or a particular embodiment of the invention, such connotation is meant to apply

throughout this specification, i.e., also in the context of other aspects or embodiments of the invention, unless otherwise defined.

In the following passages, different aspects or embodiments of the invention are defined in more detail. Each aspect or embodiment so defined may be combined with any other aspect(s) or embodiment(s) unless clearly indicated to the contrary. In particular, any feature indicated as being preferred or advantageous may be combined with any other feature or features indicated as being preferred or advantageous.

Reference throughout this specification to “one embodiment”, “an embodiment” means that a particular feature, structure or characteristic described in connection with the embodiment is included in at least one embodiment of the present invention. Thus, appearances of the phrases “in one embodiment” or “in an embodiment” in various places throughout this specification are not necessarily all referring to the same embodiment, but may. Furthermore, the particular features, structures or characteristics may be combined in any suitable manner, as would be apparent to a person skilled in the art from this disclosure, in one or more embodiments. Furthermore, while some embodiments described herein include some but not other features included in other embodiments, combinations of features of different embodiments are meant to be within the scope of the invention, and form different embodiments, as would be understood by those in the art. For example, in the appended claims, any of the claimed embodiments can be used in any combination.

The present invention is based on the observation that administration of vitamin D₃ in combination with a calcium source and a stabilized phytase to poultry improved the calcium and phosphorus regulation in the animals, thereby also improving musculoskeletal integrity and eggshell quality.

The inventors here demonstrate that by administration of a novel mixture of vitamin D₃, a calcium source and a stabilized phytase, the calcium and phosphorus regulation in the animals is synergistically improved. More specifically, by improving the calcium and phosphorus regulation, the musculoskeletal strength and integrity of the animals is improved, which contributes to the development and maintenance of a robust musculoskeletal system. After all, gait abnormalities and leg disorders are known as important issues found in poultry and egg production. These issues provide a welfare concern because lame birds may be incapable of reaching feed and water, are in pain and might become restricted in performance of natural behaviours. In broiler production systems with fast growing genetics, an increased prevalence of lameness is observed which varies from minor reduced capability to carry weight up to total immobility. Evidently, musculoskeletal problems also lead to reduced egg productivity of layer poultry.

By improving the calcium and phosphorus regulation in poultry, the compositions and methods as disclosed herein also improve the eggshell quality of the poultry. Although the process for the development of the eggshell is not yet fully known, it has nevertheless been shown that nutritional elements, in particular calcium, phosphorus and vitamin D₃, in the feed of laying poultry could have beneficial effects on the functional properties of the shell. As shown in the examples herein, the inventors have now found that administration of the combination of vitamin D₃, a stabilized phytase and calcium gluconate and calcium chloride as calcium source, reduced the presence of egg damage in layer poultry. The compositions and methods as disclosed herein therefore also provide an economic benefit by reducing the egg breakage and cracking rates and by increasing the yield.

10 An additional advantage of the compositions as disclosed herein is that the composition does not form a biofilm, which makes it particularly suitable for administration by way of the drinking water of animals. More specifically, the compositions as disclosed herein comprise no or only limited amounts of substances that may facilitate the formation of biofilms at the inner lining of drinking water systems, such as organic molecules and/or oils.

15 Accordingly, a first aspect of the application provides an orally administrable composition comprising vitamin D₃ or a derivative or analogue thereof, a calcium source and a phytase.

The compositions envisaged herein thus comprise vitamin D₃ or a derivative or analogue thereof. Vitamin D₃, also known as cholecalciferol, is the inactive form of vitamin D that can be ingested through dietary intake or be generated endogenously in the skin of animals exposed to UV light. In the liver, vitamin D₃ is converted into the active 25-hydroxyvitamin D₃ by hydroxylation, followed by conversion into 1,25-dihydroxyvitamin D₃, also known as 1,25-dihydroxycholecalciferol, calcitriol, or 12(OH)₂D₃, which is transported via the blood to its classic target organs, namely the intestine, kidney and bones. In the composition as envisaged herein, vitamin D₃, or any derivative or analogue is present. The derivatives or analogues can be selected from calcitriol, calcipotriol, or any other derivative or analogue of vitamin D₃ known in the art. In preferred embodiments, the orally administrable composition comprises vitamin D₃.

In particular embodiments, the compositions envisaged herein comprise at least 200 IU/l vitamin D₃ or a derivative or analogue thereof; particularly at least 400 IU/l vitamin D₃ or a derivative or analogue thereof. In further preferred embodiments, the compositions comprise in between 400 IU/l and 1000 IU/l vitamin D₃ or a derivative or analogue thereof; in particular in between 500 IU/l and 800 IU/l vitamin D₃ or a derivative or analogue thereof. For example, the compositions envisaged herein comprise 400 IU/l, 450 IU/l, 500 IU/l, 550 IU/l, 600 IU/l, 650 IU/l, 700 IU/l, 750 IU/l or 800 IU/l vitamin D₃ or a derivative or analogue thereof. In some embodiments, the composition comprises in between

550 IU/l and 650 IU/l vitamin D₃ or a derivative or analogue thereof, such as for example 550 IU/l, 560 IU/l, 570 IU/l, 580 IU/l, 590 IU/l, 600 IU/l, 610 IU/l, 620 IU/l, 630 IU/l, 640 IU/l or 650 IU/l vitamin D₃ or a derivative or analogue thereof.

In particular embodiments, in the preparation of the orally administrable compositions as disclosed herein, the vitamin D₃, or a derivative or analogue thereof is added to the orally administrable compositions from a stock solution comprising about 10 MIU vitamin D₃ or derivative or analogue thereof in a vegetable oil, such as for example corn oil, soybean oil or sunflower oil. Preferably, the vitamin D₃, or derivative or analogue thereof, stock solution comprises about 10 MIU vitamin D₃ or derivative or analogue thereof in soybean oil.

10 The orally administrable composition as disclosed herein also comprises a calcium source. In particular, the calcium source is an organic calcium salt or a hydrate thereof, an inorganic calcium salt or a hydrate thereof, or a combination of an inorganic and organic calcium salt, or any hydrates thereof. Inorganic calcium salts in the composition envisaged herein can be selected from the group comprising calcium chloride, calcium phosphate, calcium sulfate, calcium oxide, calcium hydroxide, and calcium carbonate, or any hydrates thereof. Preferably, the inorganic calcium salt is calcium chloride. Organic calcium salts in the composition envisaged herein can be selected from the group comprising calcium gluconate, calcium pyruvate, calcium acetate, calcium benzoate, calcium L-threonate, or any hydrates thereof. Preferably, the organic salt is calcium gluconate or calcium gluconate hydrate.

20 In particular embodiments, the compositions as disclosed herein comprise in between 5% m/v and 15% m/v of a calcium salt; preferably in between 7% m/v and 12% m/v of a calcium salt, wherein the calcium salt is an organic calcium salt, an inorganic calcium salt, or a combination of an inorganic or organic calcium salt or any hydrates thereof. For example, the compositions as disclosed herein may comprise 7% m/v, 7.5% m/v, 8% m/v, 8.5% m/v, 9% m/v, 9.5% m/v, 10% m/v, 10.5% m/v, 11% m/v, 11.5% m/v, 12% m/v, of a calcium salt that is selected from an organic calcium salt, an inorganic calcium salt, or a combination of an inorganic or organic calcium salt or any hydrates thereof.

In particular embodiments, the compositions as disclosed herein comprise an inorganic calcium salt as calcium source. In further preferred embodiments, the compositions comprise in between 5% m/v and 15% m/v of the inorganic calcium salt or hydrate thereof; in particular in between 7% m/v and 12% m/v of the inorganic calcium salt. Thus, in some embodiments, the compositions comprise 7% m/v, 7.5% m/v, 8% m/v, 8.5% m/v, 9% m/v, 9.5% m/v, 10% m/v, 10.5% m/v, 11% m/v, 11.5% m/v, 12% m/v of inorganic calcium salt or hydrate thereof. In particular embodiments, the inorganic calcium salt is selected from calcium chloride, calcium phosphate, calcium sulfate, calcium oxide, calcium

hydroxide, and calcium carbonate, or any hydrates thereof. In particular preferred embodiments, the inorganic calcium salt is calcium chloride. Thus, in particular embodiments, the inorganic calcium salt selected from calcium chloride, calcium phosphate, calcium sulphate, calcium oxide, calcium hydroxide, and calcium carbonate, or any hydrate thereof is present in between 5% m/v and 15% m/v; preferably in between 7% m/v and 12% m/v, in the composition as disclosed herein. In preferred 5 embodiments, the composition comprises calcium chloride as inorganic calcium - salt that is present in between 5% m/v and 15% m/v; preferably in between 7% m/v and 12% m/v, in the composition as disclosed herein.

In particular embodiments, the compositions as disclosed herein comprise an organic calcium salt as calcium source. In further preferred embodiments, the compositions comprise in between 5% m/v and 15% m/v of the organic calcium salt or hydrate thereof; in particular in between 7% m/v and 12% m/v of the organic calcium salt. Thus, in some embodiments, the compositions comprise 7% m/v, 7.5% m/v, 8% m/v, 8.5% m/v, 9% m/v, 9.5% m/v, 10% m/v, 10.5% m/v, 11% m/v, 11.5% m/v, 12% m/v of organic calcium salt or hydrate thereof. In particular embodiments, the organic calcium salt is selected 15 from calcium gluconate, calcium pyruvate, calcium acetate, calcium benzoate, calcium L-threonate, or any hydrates thereof. Preferably, the organic salt is calcium gluconate or calcium gluconate hydrate. Thus, in particular embodiments, the organic calcium salt selected from calcium gluconate, calcium pyruvate, calcium acetate, calcium benzoate, calcium L-threonate, or any hydrates thereof is present in between 5% m/v and 15% m/v; preferably in between 7% m/v and 12% m/v, in the composition as 20 disclosed herein.

In particular embodiments, the compositions as disclosed herein comprise a combination of an inorganic calcium salt or hydrate thereof and an organic calcium salt or hydrate thereof as calcium source. In further preferred embodiments, the compositions comprise in between 0.1% m/v and 2% m/v; preferably in between 0.3% m/v and 1% m/v, of an organic calcium salt or hydrate thereof, in combination with 4.7% m/v to 14% m/v; preferably with 4.9% m/v to 13% m/v, of a inorganic calcium 25 salt or hydrate thereof. In particular further embodiments, the composition as disclosed herein comprises 7% m/v to 12% m/v calcium chloride in combination with 0.3% m/v to 1% m/v calcium gluconate monohydrate as calcium source.

The compositions envisaged herein further comprise a phytase. In some embodiments, the phytase is 30 a fungal phytase or a bacterial phytase. In some further embodiments, the phytase is a fungal phytase derived from *A. niger* or from *Peniophora lycii*; preferably the phytase that is derived from the *A. niger* fungal strain. In some other embodiments, the phytase is derived from bacteria, such as *E. coli*, *Citrobacter braakii* or *Buttiauxella spp.* In some embodiments, the phytase is a recombinant phytase.

In some embodiments, the phytase is a recombinant phytase produced by the *Aspergillus niger* fungal strain. The bacterial or fungal phytase as used herein can be produced in several producing organisms, such as *A. niger*, *Trichoderma reesei*, *Asperigillus oryzae*, *Pichia pastoris*, or *Schizosaccharomyces pombe*. In some embodiments, the phytase is a recombinant fungal or bacterial phytase produced in a producing organism selected from *A. niger*, *Trichoderma reesei*, *Asperigillus oryzae*, *Pichia pastoris*, or *Schizosaccharomyces pombe*. In some preferred embodiments, the phytase is a fungal phytase produced in *A. niger*.

Phytate, a salt complex of myo-inositol-1,2,3,4,5,6-hexakisdihydrogenphosphate, is a phosphorus storage compound in plants, which accounts for approximately two-thirds of the total phosphorus in plant ingredients. In chicken, phytate is not well used as a source of phosphorus because of limited endogenous phytase in the digestive tract of poultry. Administration of phytase to poultry animals increases the amount of plant phosphorus available to the animals and reduces the amount of inorganic phosphorus that needs to be added to the diet, resulting in substantial savings on feed costs.

In specific embodiments, the orally administrable composition as disclosed herein comprises phytase in a range from about 10000 FTU to about 100000 FTU per kg composition, wherein FTU (phytase activity unit) is defined as the amount of phytase that liberates 1 micromole of inorganic phosphorus per minute from 0.0051 mol/l sodium phytate at 37°C and pH 5.5. In preferred embodiments, the orally administrable composition as disclosed herein comprises from phytase in a range from about 25000 FTU/kg to about 75000 FTU/kg composition, wherein FTU is defined as the amount of phytase that liberates 1 micromole of inorganic phosphorus per minute from 0.0051 mol/l sodium phytate at 37°C and pH 5.5. For example, the orally administrable compositions as disclosed herein comprises 30000 FTU/kg, 35000 FTU/kg, 40000 FTU/kg, 45000 FTU/kg, 50000 FTU/kg, 55000 FTU/kg, 60000 FTU/kg, 70000 FTU/kg or 75000 FTU/kg phytase.

In specific embodiments, the orally administrable composition as disclosed herein comprises at least 200 IU/l; preferably at least 400 IU/l vitamin D₃ or a derivative or analogue thereof; in combination with 5% m/v to 15% m/v of a calcium salt and 10000 FTU/kg to 100000 FTU/kg phytase. The inventors have found that this specific ratio of vitamin D₃, calcium salt and phytase, allows dosing at a ratio which ensures the synergistic effect between these compound without significant side-effects. More specifically, the composition comprises in between 500 IU/l and 800 IU/l vitamin D₃ or a derivative or analogue thereof, in combination with 7% m/v to 13% m/v of a inorganic and organic calcium salt, and 25000 FTU/kg to 75000 FTU/kg phytase. Even further, the composition as envisaged herein comprises in between 500 IU/l and 800 IU/l vitamin D₃ or a derivative or analogue thereof, in combination with

7% m/v to 12% m/v of an inorganic calcium salt, in particular calcium chloride, and 0.3% m/v to 1% m/v of an organic calcium salt, in particular calcium gluconate monohydrate, and 25000 FTU/kg to 75000 FTU/kg phytase.

5 The orally administered compositions as envisaged are water soluble compositions, with the vitamin D₃, the one or more calcium salts and the phytase being suspended in a liquid, in particular in water.

In particular embodiments, the compositions as envisaged herein may further comprise other nutritional or mineral elements, such as for example kalium, natrium or magnesium sources. For example, the compositions envisaged herein may comprise kalium sorbate, natrium benzoate and/or magnesium chloride, or any hydrate thereof.

10 In particular embodiments, the compositions envisaged herein comprise nitric acid.

In particular embodiments, the compositions envisaged herein comprise an emulsifier, for example a synthetic emulsifier such as glyceryl polyethylene glycol ricinoleate.

15 The composition as envisaged herein is an orally administrable composition, in particular a liquid orally administrable composition. In preferred embodiments, the composition is added to the drinking water of poultry. Other modes of administration can also be envisaged, such as for example addition of the composition to the feed. For example, the composition can be added to the feed of the poultry.

20 The inventors of the present application specifically found that the compositions as envisaged herein particularly affect and improve the calcium and phosphorus regulation in poultry, thereby improving the musculoskeletal integrity of the animals as well as the eggshell quality while limiting negative side effects. The present application therefore further provides the orally administrable composition as envisaged herein for use in the treatment and/or prevention of a disturbed calcium and phosphorus regulation in poultry. As used herein, a disturbed calcium and phosphorus regulation is to be understood as any deviation from the normal physiological levels of calcium and phosphorus in the

25 body that may lead to pathological effects and symptoms. The compositions as envisaged herein are therefore for use in the improvement and normalization of the calcium and phosphorus regulation and for normalization of the calcium and phosphorus levels in the blood of the poultry. Improvement of calcium and phosphorus regulation also refers to normalizing the absorption of dietary calcium and phosphorus by the small intestine and reabsorption of calcium by the kidneys.

30 In particular embodiments, the compositions as used herein are provided for use in preventing a reduced eggshell quality of poultry. The compositions are further provided for reinforcing the eggshell

of poultry. Indeed, by improving the calcium and phosphorus regulation in the animals, the compositions as disclosed herein reinforce the eggshells of the poultry, thereby improving their quality. The compositions as disclosed herein can also be applied in a preventive setting, used for preventing a decreased in the eggshell quality. Due to their effect on the eggshell quality and strength,
5 the compositions are particularly useful to improve the mechanical resistance of the eggs of the poultry, thereby reducing the egg breakage rates and egg cracking rates.

In a further aspect, a method for maintaining a normal calcium and phosphorus regulation in poultry is provided, said method comprising administering to the poultry the composition as envisaged herein. As used herein, maintaining a normal calcium and phosphorus regulation is to be understood as
10 maintaining normal physiological levels of calcium and phosphorus in the body and maintaining the physiological absorption levels of dietary calcium and phosphorus by the small intestine and reabsorption levels of calcium by the kidneys.

In another aspect, a method for maintaining and/or improving the quality of the eggshell of poultry is disclosed as well as a method for preventing a reduced quality of the eggshell of poultry, wherein the
15 compositions according to any of the embodiments as disclosed herein is administered to the poultry.

By administering the compositions as envisaged herein to the poultry, in particular to laying poultry, it is possible to reinforce eggshells and to lower the egg breakage rate and the egg cracking rate compared with the case of not administering the same. The compositions according to the invention may administered to any birds without restriction. They can be added to the feed or to the drinking
20 water of the animals. Preferably, the compositions as envisaged herein are added to the drinking water of the poultry.

The compositions as envisaged herein are thus typically provided as orally administrable compositions, meaning that they can be administered orally to the animals. Accordingly, the compositions as disclosed herein are liquid compositions.

In particular embodiments, the compositions as envisaged herein are thus administered via the
25 drinking water of the poultry. By administering the composition via the drinking water, the animals have ad libitum access to the composition. In some particular embodiments, the compositions as envisaged herein are administered via the drinking water of the poultry in a dilution of from 0.1 to 10% v/v; more preferably in a dilution of from 0.01% to 1% v/v.

The compositions may be administered once daily or multiple times daily, for example two, three, or
30 four times per day. In particular and preferred embodiments, the compositions are provided continuously and ad libitum to the animals, in particular via the drinking water.

In particular embodiments, the compositions as disclosed herein are ad libitum administered to the poultry during at least 3 consecutive days, such as during 3 days, during 4 days, during 5 days, during 6 days, during 7 days, or even during more than 7 days.

5 In particular embodiments, the compositions as disclosed herein are administered to the poultry for at least 3 consecutive days per week; preferably for 3 to 7 consecutive days per week. For example, the compositions as disclosed herein can be administered for 3 days, followed by a 4-days period without administration of the composition, or for 4 days followed by a 3-days period without administration of the composition, or for 5 days followed by a 2-days period without administration of the composition, or for 6 days followed by one day without administration of the composition. This
10 treatment regimen can further be applied for 1 week, for 2 weeks, for 3 weeks, for 4 weeks, or even for more than 4 weeks.

During periods of challenge, such as disturbed calcium and phosphorus regulation or moderately or severely reduced eggshell quality, it is envisaged to administer the compositions as disclosed herein for several consecutive days, preferably for at least 3 consecutive days, such as for 3 to 7 consecutive
15 days. In particular embodiments, the compositions may be administered for several weeks.

In particular embodiments, when the compositions are intended to be administered in a preventive setting, such as to maintain normal calcium and phosphorus blood levels, or to maintain the eggshell quality of the poultry, compositions are administered once a week for one, two, three, four or even more than four weeks. In other particular embodiments, the compositions as envisaged herein may
20 be administered in a preventive setting for 3 to 7 days a week, such as for example in broilers.

The term "poultry" as used in the present application refers to chickens, home-bred chickens, silk fowls, turkeys, wild ducks, quails, ducks, guinea fowls, pheasants, crossbreed of wild and domestic ducks, geese, ostriches, and the like. The present invention is preferably applied to chickens, ducks and/or quails; even more preferably to chickens.

25 In particular embodiments, the poultry is selected from layer poultry, parent stock or broilers. In some further preferred embodiments, the poultry is selected from layer poultry, parent stock or broilers of chicken, ducks and/or quails. In still some further embodiments, the poultry is selected from layer chicken, parent stock chicken and/or broiler chickens. In some preferred embodiments, the invention is applied to laying hens bred for the purpose of producing chicken eggs. There is no particular
30 limitation as to the kinds of chickens.

It is apparent that there have been provided in accordance with the invention products, methods, and uses, that provide for substantial advantages as set forth above. While the invention has been

described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications, and variations will be apparent to those skilled in the art in light of the foregoing description. Accordingly, it is intended to embrace all such alternatives, modifications, and variations as follows in the spirit and broad scope of the appended claims.

- 5 The above aspects and embodiments are further supported by the following non-limiting examples.

EXAMPLES

Materials and methods

10 The percentage of damaged eggs was evaluated in two separate flocks of a commercial layer farm, each comprising 14000 laying hens per house. Laying hens were from the Novagen breed, and during treatment they had an age in between 47 to 65 weeks. The hens were housed in a caged laying system with 14000 hens per house.

15 In one housing system, the flock of laying hens was only administered the normal drinking water. In the other housing system, the laying hens received the composition according to the invention in their drinking water. More specifically, the composition comprising vitamin D₃ (400-600 IU/L), 5% m/v to 15% m/v of a calcium source comprising calcium chloride and calcium gluconate monohydrate and 25000 FTU/kg to 75000 FTU/kg phytase was administered to the laying hens in a dilution of 0.1% in their drinking water, and the composition was administered for 3 consecutive days per week during 6 weeks (starting at week 60, indicated by the arrow in Figure 1).

20

Results

25 As can be observed in Figure 1, the percentage of damaged eggs rises at an average rate of about 0.2% every week. However, administration of the composition as used herein reduced the percentage of damaged eggs by about 0.2-0.4% according to the trend for this flock. Remarkably, at the start of the administration of the composition (black arrow in Figure 1), the flock had an average age of about 60 weeks, concluding that the composition reduced the eggshell damage even in animals that are becoming older.

30 When the percentage of damaged eggs in the treated hens was compared to the hens that only received normal drinking water, also a reduction of egg damage could be observed (Figure 2). More specifically, the average percentage of damaged eggs in the control group after 11 days is 1.63%,

whereas the average percentage of damaged eggs in the treated group after 11 days was 1.22%, showing a difference of 0.41% in the damaged egg rates.

CONCLUSIES

1. Een oraal toedienbare samenstelling omvattende vitamine D₃ of een afgeleide stof of analoog hiervan, een calcium bron en een fytase.
5
2. De samenstelling volgens conclusie 1 waarbij de samenstelling ten minste 200 IU/l vitamine D₃ of een afgeleide stof of analoog hiervan bevat; bij voorkeur ten minste 400 IU/l vitamine D₃ of een afgeleide stof of analoog hiervan.
- 10 3. De samenstelling volgens conclusie 1 of 2 waarbij de calcium bron een organisch calcium zout, een anorganisch calcium zout of een combinatie van een organisch en een anorganisch calcium zout is.
- 15 4. De samenstelling volgens conclusie 3 waarbij het organisch calcium zout geselecteerd wordt uit de groep omvattende calcium chloride, calcium fosfaat, calcium sulfaat, calcium oxide, calcium hydroxide en calcium carbonaat, of een hydraat hiervan.
- 20 5. De samenstelling volgens conclusie 3 waarbij het organisch calcium zout geselecteerd wordt uit de groep omvattende calcium gluconaat, calcium pyruvaat, calcium acetaat, calcium benzoaat, calcium L-threonaat, of een hydraat hiervan.
- 25 6. De samenstelling volgens eender welke voorgaande conclusie waarbij de calcium bron een combinatie is van een organisch calcium zout en een anorganisch calcium zout, of een hydraat hiervan; bij voorkeur een combinatie van calcium chloride en calcium gluconaat monohydraat.
- 30 7. De samenstelling volgens eender welke voorgaande conclusie waarbij het fytase afkomstig is van de *Aspergillus niger* schimmel.
8. De samenstelling volgens eender welke voorgaande conclusie, voor gebruik in de behandeling en/of preventie van een verstoorde calcium en fosfor regulatie in gevogelte.
9. De samenstelling volgens eender welke voorgaande conclusies 1 tot 7 voor gebruik in de preventie van een verminderde eierschaalkwaliteit in gevogelte.

10. Een methode voor het behoud van een normale calcium en fosfor regulatie in gevogelte, deze methode omvattende het toedienen van een samenstelling volgens eender welke voorgaande conclusie 1 tot 7 aan gevogelte.
- 5 11. Een methode voor het behoud en/of de verbetering van de eierschaalkwaliteit in gevogelte en/of het verhinderen van een verminderde eierschaalkwaliteit in gevogelte, deze methode omvattende het toedienen van een samenstelling volgens eender welke voorgaande conclusie 1 tot 7 aan gevogelte.
- 10 12. De samenstelling voor gebruik volgens conclusies 8 of 9, of de methode volgens conclusies 10 of 11 waarbij de samenstelling toegediend wordt via het drinkwater van het gevogelte; bij voorkeur waarbij de samenstelling toegediend wordt via het drinkwater van het gevogelte in een verdunning van 0.1% tot 10% v/v; nog meer bij voorkeur in een verdunning van 0.01% tot 1% v/v.
- 15 13. De samenstelling voor gebruik volgens één van de conclusies 8, 9 of 12, of de methode volgens één van de conclusies 10 tot 12, waarin de samenstelling *ad libitum* wordt aangeboden aan het gevogelte gedurende ten minste 3 opeenvolgende dagen; bij voorkeur waarbij de samenstelling *ad libitum* wordt aangeboden gedurende 3 tot 7 opeenvolgende dagen; nog meer bij voorkeur waarbij de samenstelling *ad libitum* wordt aangeboden gedurende 3 tot 7 opeenvolgende dagen per week.
- 20 14. De samenstelling volgens één van de conclusies 1 tot 7, de samenstelling voor gebruik volgens één van de conclusies 8, 9, 12 of 13, of de methode volgens één van de conclusies 10 tot 13, waarbij het gevogelte geselecteerd wordt uit leghennen, kweek gevogelte, of mestkuikens.
- 25 15. De samenstelling volgens één van de conclusies 1 tot 7 of 14, de samenstelling voor gebruik volgens één van de conclusies 8, 9, 12, 13 of 14, of de methode volgens één van de conclusies 10 tot 14, waarbij het gevogelte geselecteerd wordt uit kippen, eenden en kwartels.

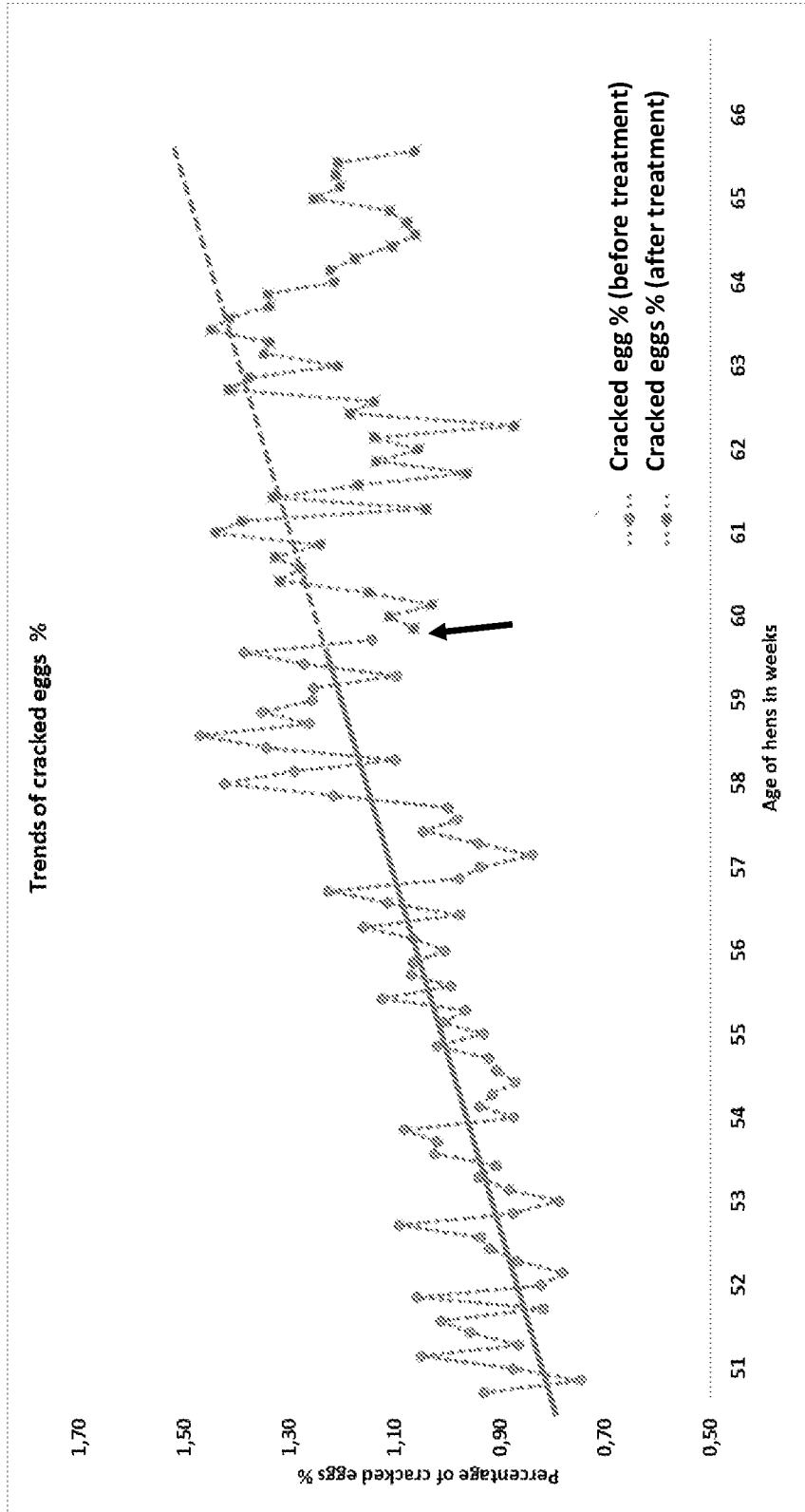


Fig. 1

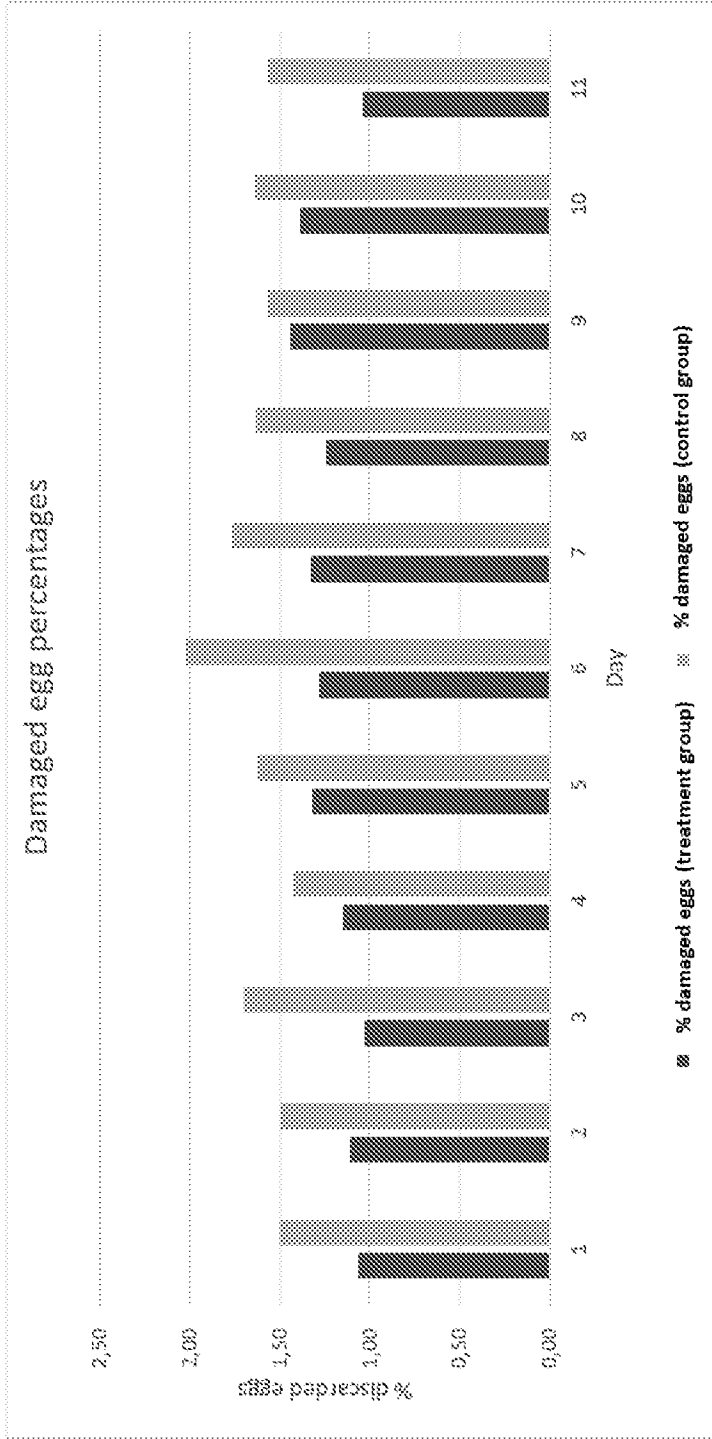


Fig. 2

SAMENWERKINGSVERDRAG (PCT)

RAPPORT BETREFFENDE NIEUWHEIDSONDERZOEK VAN INTERNATIONAAL TYPE

IDENTIFICATIE VAN DE NATIONALE AANVRAGE	KENMERK VAN DE AANVRAGER OF VAN DE GEMACHTIGDE
Nederlands aanvraag nr. 2028362	Indieningsdatum 01-06-2021
	Ingeroepen voorrangdatum
Aanvrager (Naam) Intracare BV	
Datum van het verzoek voor een onderzoek van internationaal type 14-08-2021	Door de Instantie voor Internationaal Onderzoek aan het verzoek voor een onderzoek van internationaal type toegekend nr. SN79334
I. CLASSIFICATIE VAN HET ONDERWERP (bij toepassing van verschillende classificaties, alle classificatiesymbolen opgeven)	
Volgens de internationale classificatie (IPC) Zie onderzoeksrapport	
II. ONDERZOCHE GEBIEDEN VAN DE TECHNIEK	
Onderzochte minimumdocumentatie	
Classificatiesysteem	Classificatiesymbolen
IPC	Zie onderzoeksrapport
Onderzochte andere documentatie dan de minimum documentatie, voor zover dergelijke documenten in de onderzochte gebieden zijn opgenomen	
III.	GEEN ONDERZOEK MOGELIJK VOOR BEPAALDE CONCLUSIES (opmerkingen op aanvullingsblad)
IV.	GEBREK AAN EENHEID VAN UITVINDING (opmerkingen op aanvullingsblad)

**ONDERZOEKSRAPPORT BETREFFENDE HET
RESULTAAT VAN HET ONDERZOEK NAAR DE STAND
VAN DE TECHNIEK VAN HET INTERNATIONALE TYPE**

Nummer van het verzoek om een onderzoek naar
de stand van de techniek
NL 2028362

<p>A. CLASSIFICATIE VAN HET ONDERWERP</p> <p>INV. A61K31/593 A61K33/06 A61K33/08 A61K33/10 A61K38/43</p> <p>A23K20/174 A23K20/189 A23K20/24</p> <p>ADD.</p> <p>Volgens de Internationale Classificatie van octrooien (IPC) of zowel volgens de nationale classificatie als volgens de IPC.</p>		
<p>B. ONDERZOCHETE GEBIEDEN VAN DE TECHNIEK</p> <p>Onderzochte minimum documentatie (classificatie gevolgd door classificatiesymbolen)</p> <p>A61K C11C A23K</p>		
<p>Onderzochte andere documentatie dan de minimum documentatie, voor dergelijke documenten, voor zover dergelijke documenten in de onderzochte gebieden zijn opgenomen</p>		
<p>Tijdens het onderzoek geraadpleegde elektronische gegevensbestanden (naam van de gegevensbestanden en, waar uitvoerbaar, gebruikte trefwoorden)</p> <p>EPO-Internal, WPI Data</p>		
<p>C. VAN BELANG GEACHTE DOCUMENTEN</p>		
<p>Categorie °</p>	<p>Geciteerde documenten, eventueel met aanduiding van speciaal van belang zijnde passages</p>	<p>Van belang voor conclusie nr.</p>
<p>X</p>	<p>KESHAVARZ K: "A comparison between cholecalciferol and 25-OH-cholecalciferol on performance and eggshell quality of hens fed different levels of calcium and phosphorus", POULTRY SCIENCE, OXFORD UNIVERSITY PRESS, OXFORD, deel 82, nr. 9, 31 augustus 2003 (2003-08-31), bladzijden 1415-1422, XP009533003, ISSN: 0032-5791, DOI: 10.1093/PS/82.9.1415 * het gehele document * * tabel 6 *</p> <p style="text-align: center;">----- -/--</p>	<p>1-4, 6, 8-11, 13-15</p>
<p><input checked="" type="checkbox"/> Verdere documenten worden vermeld in het vervolg van vak C. <input checked="" type="checkbox"/> Leden van dezelfde octroofamilie zijn vermeld in een bijlage</p>		
<p>° Speciale categorieën van aangehaalde documenten</p> <p>"A" niet tot de categorie X of Y behorende literatuur die de stand van de techniek beschrijft</p> <p>"D" in de octrooiaanvraag vermeld</p> <p>"E" eerdere octrooi(aanvraag), gepubliceerd op of na de indieningsdatum, waarin dezelfde uitvinding wordt beschreven</p> <p>"L" om andere redenen vermelde literatuur</p> <p>"O" niet-schriftelijke stand van de techniek</p> <p>"P" tussen de voorrangsdatum en de indieningsdatum gepubliceerde literatuur</p> <p>"T" na de indieningsdatum of de voorrangsdatum gepubliceerde literatuur die niet bezwarend is voor de octrooiaanvraag, maar wordt vermeld ter verheldering van de theorie of het principe dat ten grondslag ligt aan de uitvinding</p> <p>"X" de conclusie wordt als niet nieuw of niet inventief beschouwd ten opzichte van deze literatuur</p> <p>"Y" de conclusie wordt als niet inventief beschouwd ten opzichte van de combinatie van deze literatuur met andere geciteerde literatuur van dezelfde categorie, waarbij de combinatie voor de vakman voor de hand liggend wordt geacht</p> <p>"&" lid van dezelfde octroofamilie of overeenkomstige octrooipublicatie</p>		
<p>Datum waarop het onderzoek naar de stand van de techniek van internationaal type werd voltooid</p> <p>27 januari 2022</p>		<p>Verzenddatum van het rapport van het onderzoek naar de stand van de techniek van internationaal type</p>
<p>Naam en adres van de instantie</p> <p>European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016</p>		<p>De bevoegde ambtenaar</p> <p>Jakobs, Andreas</p>

**ONDERZOEKSRAPPORT BETREFFENDE HET
RESULTAAT VAN HET ONDERZOEK NAAR DE STAND
VAN DE TECHNIEK VAN HET INTERNATIONALE TYPE**

Nummer van het verzoek om een onderzoek naar
de stand van de techniek

NL 2028362

C.(Vervolg). VAN BELANG GEACHTE DOCUMENTEN			
Categorie °	Geciteerde documenten, eventueel met aanduiding van speciaal van belang zijnde passages	Van belang voor conclusie nr.	
X	CN 105 746 938 A (BEIJING HUADU YUKOU POULTRY IND CO LTD) 13 juli 2016 (2016-07-13) * het gehele document * * conclusies 1-7 *	1-4, 6, 8-11, 13-15	

X	CN 105 360 694 A (JINAN HEMEIHUA FEED CO LTD) 2 maart 2016 (2016-03-02) * het gehele document * * conclusies 1-7,10 *	1-4, 6, 8-11, 13-15	

X	CN 104 106 740 A (ANHUI QIANGYING DUCK INDUSTRY GROUP CO LTD) 22 oktober 2014 (2014-10-22) * het gehele document * * conclusies 1-3 *	1-4, 8, 10,13-15	

X	CN 103 931 908 A (SICHUAN TQLS INDUSTRY CO LTD) 23 juli 2014 (2014-07-23) * het gehele document * * conclusies 1-6 *	1-4, 6, 8-11, 13-15	

X	CN 102 919 601 A (INNER MONGOLIA SAIFEIYA AGRICULTURAL SCIENCE AND TECHNOLOGY DEV CO LTD) 13 februari 2013 (2013-02-13) * het gehele document * * conclusies 1-2 *	1-4, 6, 8, 10,13-15	

X	CN 107 801 863 A (JIANGSU ZHONGMEI CHANGJIANG BIOTECHNOLOGY CO LTD) 16 maart 2018 (2018-03-16) * het gehele document * * conclusies 1-4; voorbeeld 1 *	1-4, 6, 8-11, 13-15	

X	CN 101 485 398 A (SHANDONG ZHONGJI ANIMAL HUSBAN [CN]) 22 juli 2009 (2009-07-22) * het gehele document * * conclusies 1-4; voorbeelden 1-3 *	1-4, 6, 8, 10,13-15	

X	CN 101 485 399 A (SHANDONG ZHONGJI ANIMAL HUSBAN [CN]) 22 juli 2009 (2009-07-22) * het gehele document * * conclusies 1-3 *	1-4, 6, 8, 10,13-15	

X	CN 103 431 170 A (YICHENG DAZHONG FEED CO LTD) 11 december 2013 (2013-12-11) * het gehele document * * conclusies 1-3 *	1-4, 6, 8, 10,13-15	

15	X	ZA 200 504 594 B (PHARMACHOICE HEALTHCARE PTY LT) 26 juli 2006 (2006-07-26) * het gehele document * * conclusies 1-21 *	1-4, 6, 7

1		-/--	

**ONDERZOEKSRAPPORT BETREFFENDE HET
 RESULTAAT VAN HET ONDERZOEK NAAR DE STAND
 VAN DE TECHNIEK VAN HET INTERNATIONALE TYPE**

Nummer van het verzoek om een onderzoek naar
 de stand van de techniek
NL 2028362

C.(Vervolg). VAN BELANG GEACHTE DOCUMENTEN		
Categorie °	Geciteerde documenten, eventueel met aanduiding van speciaal van belang zijnde passages	Van belang voor conclusie nr.
X	<p>US 5 366 736 A (EDWARDS JR HARDY M [US]) 22 november 1994 (1994-11-22) * het gehele document * * conclusies 1,3,6,8; tabel 3b *</p> <p style="text-align: center;">-----</p>	1-4, 8
X	<p>FARIDI A. ET AL: "A meta-analysis of the effects of nonphytate phosphorus on broiler performance and tibia ash concentration", POULTRY SCIENCE, deel 94, nr. 11, 1 november 2015 (2015-11-01), bladzijden 2753-2762, XP055884163, Oxford ISSN: 0032-5791, DOI: 10.3382/ps/pev280 * het gehele document *</p> <p style="text-align: center;">-----</p>	1-4, 6, 8, 10, 13-15

**ONDERZOEKSRAPPORT BETREFFENDE HET
RESULTAAT VAN HET ONDERZOEK NAAR DE STAND
VAN DE TECHNIEK VAN HET INTERNATIONALE TYPE**

Informatie over leden van dezelfde octrooifamilie

Nummer van het verzoek om een onderzoek naar
de stand van de techniek

NL 2028362

In het rapport genoemd octrooigeschrift	Datum van publicatie	Overeenkomend(e) geschrift(en)	Datum van publicatie
CN 105746938	A	13-07-2016	GEEN
CN 105360694	A	02-03-2016	GEEN
CN 104106740	A	22-10-2014	GEEN
CN 103931908	A	23-07-2014	GEEN
CN 102919601	A	13-02-2013	GEEN
CN 107801863	A	16-03-2018	GEEN
CN 101485398	A	22-07-2009	GEEN
CN 101485399	A	22-07-2009	GEEN
CN 103431170	A	11-12-2013	GEEN
ZA 200504594	B	26-07-2006	GEEN
US 5366736	A	22-11-1994	US 5366736 A 22-11-1994 US 5516525 A 14-05-1996

WRITTEN OPINION

File No. SN79334	Filing date (<i>day/month/year</i>) 01.06.2021	Priority date (<i>day/month/year</i>)	Application No. NL2028362
International Patent Classification (IPC) INV. A61K31/593 A61K33/06 A61K33/08 A61K33/10 A61K38/43 A23K20/174 A23K20/189 A23K20/24			
Applicant Intracare BV			

This opinion contains indications relating to the following items:

- Box No. I Basis of the opinion
- Box No. II Priority
- Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- Box No. IV Lack of unity of invention
- Box No. V Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- Box No. VI Certain documents cited
- Box No. VII Certain defects in the application
- Box No. VIII Certain observations on the application

	Examiner Jakobs, Andreas
--	-----------------------------

WRITTEN OPINION**Box No. I Basis of this opinion**

1. This opinion has been established on the basis of the latest set of claims filed before the start of the search.
2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the application and necessary to the claimed invention, this opinion has been established on the basis of:
 - a. type of material:
 - a sequence listing
 - table(s) related to the sequence listing
 - b. format of material:
 - on paper
 - in electronic form
 - c. time of filing/furnishing:
 - contained in the application as filed.
 - filed together with the application in electronic form.
 - furnished subsequently for the purposes of search.
3. In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
4. Additional comments:

Box No. V Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty	Yes: Claims	5, 12
	No: Claims	1-4, 6-11, 13-15
Inventive step	Yes: Claims	
	No: Claims	1-15
Industrial applicability	Yes: Claims	1-15
	No: Claims	

2. Citations and explanations

see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1 Reference is made to the following documents:

- D1 KESHAVARZ K: "A comparison between cholecalciferol and 25-OH-cholecalciferol on performance and eggshell quality of hens fed different levels of calcium and phosphorus", POULTRY SCIENCE, OXFORD UNIVERSITY PRESS, OXFORD, deel 82, nr. 9, 31 augustus 2003 (2003-08-31), bladzijden 1415-1422, XP009533003, ISSN: 0032-5791, DOI: 10.1093/PS/82.9.1415
- D2 CN 105 746 938 A (BEIJING HUADU YUKOU POULTRY IND CO LTD) 13 juli 2016 (2016-07-13)
- D3 CN 105 360 694 A (JINAN HEMEIHUA FEED CO LTD) 2 maart 2016 (2016-03-02)
- D4 CN 104 106 740 A (ANHUI QIANGYING DUCK INDUSTRY GROUP CO LTD) 22 oktober 2014 (2014-10-22)
- D5 CN 103 931 908 A (SICHUAN TQLS INDUSTRY CO LTD) 23 juli 2014 (2014-07-23)
- D6 CN 102 919 601 A (INNER MONGOLIA SAIFEIYA AGRICULTURAL SCIENCE AND TECHNOLOGY DEV CO LTD) 13 februari 2013 (2013-02-13)
- D7 CN 107 801 863 A (JIANGSU ZHONGMEI CHANGJIANG BIOTECHNOLOGY CO LTD) 16 maart 2018 (2018-03-16)
- D8 CN 101 485 398 A (SHANDONG ZHONGJI ANIMAL HUSBAN [CN]) 22 juli 2009 (2009-07-22)
- D9 CN 101 485 399 A (SHANDONG ZHONGJI ANIMAL HUSBAN [CN]) 22 juli 2009 (2009-07-22)
- D10 CN 103 431 170 A (YICHENG DAZHONG FEED CO LTD) 11 december 2013 (2013-12-11)
- D11 ZA 200 504 594 B (PHARMACHOICE HEALTHCARE PTY LT) 26 juli 2006 (2006-07-26)

D12 US 5 366 736 A (EDWARDS JR HARDY M [US]) 22 november 1994
(1994-11-22)

D13 FARIDI A. ET AL: "A meta-analysis of the effects of nonphytate
phosphorus on broiler performance and tibia ash concentration",
POULTRY SCIENCE, deel 94, nr. 11, 1 november 2015 (2015-11-01),
bladzijden 2753-2762, XP055884163, Oxford ISSN: 0032-5791, DOI:
10.3382/ps/pev280

2 The present application does not meet the criteria of patentability, because the
subject-matter of claim 1-4, 6-11, 13-15 is not new.

2.1 D1 discloses the subject-matter of claims 1-4, 6, 8-11, 13-15, i.e. that two
experiments were conducted to determine whether the presence of 25-OH-
cholecalciferol (25-OH-D3) as compared to vitamin D3 produces any beneficial
effect on shell quality of laying hens. Experiment 1 consisted of a 4 x 2 factorial
arrangement of the treatments with four determined Ca levels (3.34, 4.3, 4.73,
and 4.94%) and two sources of vitamin D (vitamin D3 and 25-OH-D3, which
were used at 69 microg/kg diet or 2,760 IU/kg diet). Experiment 2 consisted of a
3 x 2 x 2 factorial arrangement of the treatments with three determined levels of
nonphytate P (NPP) (0.11, 0.21, and 0.41%), two levels of phytase (0 and 300
U/kg diet), and two sources of vitamin D (vitamin D3 and 25-OH-D3, which were
used at 69 microg/kg diet, the equivalent of 2,760 IU/kg diet). Substitution of
vitamin D3 with 25-OH-D3 in neither of the experiments produced any beneficial
effect on shell quality. A Ca level of 3.34%, which provided the birds with 3.63 g
Ca/hen per d, was adequate for performance and eggshell quality. The NPP
level of 0.11% was not sufficient to support performance. However, a NPP level
of 0.21% was adequate and resulted in performance that was comparable to
that of birds fed the 0.41% NPP diet. The presence of phytase did not have an
effect on performance but reduced several indices of the shell quality. In
conclusion, under the conditions of the current experiments, the use of 25-OH-
D3 did not provide any advantage for shell quality or production performance.
Accordingly, the combination of Calcium, Vitamin D3 and Phytase to improve
quality of egg shell in poultry is not novel.

2.2 D2 discloses the subject-matter of claims 1-4, 6, 8-11, 13-15, i.e. a poultry feed
comprising a.o. 150-190 pts. wt. calcium hydrophosphate, 60-70 pts. wt. sodium
chloride, 10-20 pts. wt. sodium bicarbonate, 2-4 pts. wt. phytase, and 8-10,000
IU vitamin D3 and avoids lack of calcium and phosphorus causing soft-shell
eggs and chicken paralysis and hypercalcemia caused by kidney swelling and
intractable diarrhea.

- 2.3 D3 discloses the subject-matter of claims 1-4, 6, 8-11, 13-15, i.e. a feed for improving eggshell color and eggshell quality of laying duck contains 120-140 pts. wt. or 120 pts. wt. calcium hydrophosphate, 8-10 pts. wt. or 8 pts. wt. composite vitamin composed of 4 %-8 % vitamin A, 0.5-2 % vitamin D3, 2-5 pts. wt. or 5 pts. wt. phytase, and other ingredients.
- 2.4 D4 discloses the subject-matter of claims 1-4, 8, 10, 13-15, i.e. premix for chicken and laying duck comprising a.o. 1 pts. wt. phytase, 90 pts. wt. calcium phosphate, 125 pts. wt. stone powder, 27 pts. wt. edible salt, 5 pts. wt. methionine, and multidimensional composite comprises 1-2 mg vitamin A, 4-5 mg vitamin D3, 0.3-1.2 mg vitamin E, 30.5-1 g vitamin K, 0.5-1.2 g riboflavin, 2-3 mg vitamin B1, 2-4 mg vitamin B2, 1-3 mg vitamin B6, 1-3 mg vitamin B12, 0.02-0.03 μ g biotin, 0.05-0.15 g folic acid, 8-10 g nicotinic acid, and 2-3 g pantothenic acid.
- 2.5 D5 discloses the subject-matter of claims 1-4, 6, 8-11, 13-15, i.e. a feed useful for improving quality and production of chicken egg and increasing thickness of the egg shell and eggshell quality. The feed comprises 48.10 pts. wt. corn, 12.88 pts. wt. soybean, 10 pts. wt. wheat, 6 pts. wt. corn germ cake, 4 pts. wt. clover powder, 5.2 pts. wt. calcium tablets, 5 pts. wt. lees, 3.5 pts. wt. calcium, 3 pts. wt. corn protein powder, 0.74 pts. wt. calcium hydrogen phosphate, 0.16 pts. wt. bentonite, 0.25 pts. wt. vegetable oils, 0.0006 pts. wt. betaine, 0.3 pts. wt. salt, 0.306 pts. wt. lysine, 0.15 pts. wt. vitamin composite, 0.1 pts. wt. choline, 0.1 pts. wt. trace element composite, 0.1 pts. wt. sodium bicarbonate, 0.109 pts. wt. methionine, and 0.005 pts. wt. phytase. Preferred Components: The vitamin composite comprises 6 pts. wt. vitamin A, 1.2 pts. wt. vitamin D3, 1.5 pts. wt. vitamin K3, 15 pts. wt. vitamin E, 1.2 pts. wt. vitamin B1, 2.5 pts. wt. vitamin B2, 0.8 pts. wt. vitamin B6, 0.5 pts. wt. vitamin B12, 0.8 pts. wt. nicotinic acid, 0.8 pts. wt. folic acid, 4.8 pts. wt. calcium pantothenate, and 5 pts. wt. biotin.
- 2.6 D6 discloses the subject-matter of claims 1-4, 6, 8, 10, 13-15, i.e. a feed composition for laying Duck comprising a.o. 0.7137 wt.% calcium hydrophosphate, 0.5000 wt.% additive, 0.3100 wt.% edible salt, 0.2000 wt.% choline, 0.1211 wt.% methionine, 0.0949 wt.% lysine, 0.0100 wt.% phytase and 0.1000 wt.% sodium bicarbonate, where the additive comprises 8 g iron, 16 g zinc, 2.4 g copper, 16 g manganese, 0.48 g iodine, 40 mg selenium, 2.4 IU vitamin A, 0.64 hundred million IU vitamin D3.
- 2.7 D7 discloses the subject-matter of claims 1-4, 6, 8-11, 13-15, i.e. a feed that improves the egg shell color, enhances the eggshell strength, reduces egg damaging rate and improves the economic benefit of breeding. The feed

- comprises a.o. 22 pts. wt. bean pulp, 5 pts. wt. corn, 15 pts. wt. calcium phosphate, 0.5 pts. wt. phytase, and laying chicken multi-vitamins including 10-200000 IU/kg vitamin A, 34-70000 IU vitamin D.
- 2.8 D8 discloses the subject-matter of claims 1-4, 6, 8, 10, 13-15, i.e. a premix for the laying hen comprising (kg) manganese sulfate (3-9), zinc sulfate (3-7), ferrous sulfate (3-7), copper sulfate (0.6-1.4), choline chloride (12-18), lysine (7-11), methionine (26-34), phytase (1.6-2.4), calcium carbonate (400-461), calcium bicarbonate (80-120), calcium pantothenate, fish meal (300-360), salt (45-75), and vitamins including 60 g of Vitamin D.
- 2.9 D9 discloses the subject-matter of claims 1-4, 6, 8, 10, 13-15, i.e. a compound premix for laying quail, comprising manganese sulfate, zinc sulfate, ferrous sulfate, copper sulfate, choline chloride, lysine, methionine, phytase, calcium carbonate, calcium hydrogen carbonate, fish meal, salt and vitamin D3
- 2.10 D10 discloses the subject-matter of claims 1-4, 6, 8, 10, 13-15, i.e. a feed, useful for egg laying hen, comprises corn, cortex phellodendri, wheat bran, stone powder, calcium hydrophosphate, methionine, lysine, phytase, salt and premix, where the premix comprises vitamin D and vitamin D3.
- 2.11 D11 discloses the subject-matter of claims 1-4, 6, 7, i.e. an oral composition for treating and preventing osteoporosis, comprises sources of calcium and magnesium, vitamin K and vitamin D and phytase (from *Aspergillus niger*).
- 2.12 D12 discloses the subject-matter of claims 1-4, 8, i.e. a composition comprising Vitamin D3, a calcium source (e.g. DiCalcium Phosphate) and phytase to be administered to poultry.
- 2.13 D13 discloses the subject-matter of claims 1-4, 6, 8, 10, 13-15, i.e. that decreasing feed costs while maintaining broiler performance at a high level with minimal environmental pollution has become a major challenge for poultry nutritionists in recent years. In this regard, phosphorus (P) is a nutrient that is problematic. To overcome this, a comprehensive knowledge of the responses of broilers to P is needed and the factors that affect its utilization need better understanding. For this purpose, a meta-analysis was conducted using results published in the literature on the responses of broilers to different levels of nonphytate P (NPP), calcium (Ca), microbial phytase (MP), and vitamin D3 or its metabolites (VD). The effects of Ca, MP, and VD on NPP requirements were investigated. Results showed significant ($P \leq 0.0001$) linear and quadratic effects of NPP on all the responses, viz. average daily gain (ADG), feed intake (FI), feed efficiency (FE), and tibia ash concentration (TA). Results showed the negative effect of high Ca levels on all investigated responses, although these

deleterious effects were alleviated when levels of NPP were increased or MP and/or VD added. Synergistic effects of MP and VD on FI and TA were observed. Best performance for all responses was found when MP and VD were added to low or moderate levels of Ca and NPP. Optimization showed higher levels of NPP are required to maximize TA compared to ADG, FI, and FE. Based on our analysis, requirements for NPP were affected mostly by Ca (increased) and MP (decreased), and, to a lesser extent, VD (inconsistent).

- 3 Dependent claims 5,12 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of novelty and/or inventive step.
- 4 The present application does not meet the criteria of patentability, because the subject-matter of claim 5, 12 does not involve an inventive step.
Claim 5: In the absence of a demonstrated effect, the use of an organic calcium salt selected from the list consisting of gluconate, pyruvate, acetate, benzoate or L-threonate are mere obvious alternatives.
Claim 12: In the absence of a demonstrated effect, the administration of the claimed composition through the drinking water instead of the feed is are mere obvious alternative.