(19) DANMARK

(10) DK/EP 1993375 T3

(12) Oversættelse af europæisk patentskrift

Patent- og Varemærkestyrelsen

(51) Int.Cl.: *A 23 K 20/111 (2016.01)* *A 23 K 50/30 (2016.01)*

(45) Oversættelsen bekendtgjort den: **2017-07-17**

(80) Dato for Den Europæiske Patentmyndigheds bekendtgørelse om meddelelse af patentet: **2017-04-12**

(86) Europæisk ansøgning nr.: **07703528.5**

(86) Europæisk indleveringsdag: **2007-02-21**

(87) Den europæiske ansøgnings publiceringsdag: **2008-11-26**

(86) International ansøgning nr.: **EP2007001471**

(87) Internationalt publikationsnr.: **WO2007104407**

(30) Prioritet: **2006-03-10 EP 06004899**

(84) Designerede stater: **AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IS IT LI LT LU LV MC NL PL PT RO SE SI SK TR**

(73) Patenthaver: **DSM IP Assets B.V., Het Overloon 1, 6411 TE Heerlen, Holland**

(72) Opfinder: **GUGGENBUHL, Patrick, 15, rue des Marronniers, F-68127 Sainte-Croix-en-Plaine, Frankrig**
Simoes-Nunes, Carlos, 10 Rue de la Liberte, F-68128 Village-Neuf, Frankrig

(74) Fuldmægtig i Danmark: **RWS Group, Europa House, Chiltern Park, Chiltern Hill, Chalfont St Peter, Bucks SL9 9FG, Storbritannien**

(54) Benævnelse: **HIDTIL UKENDT ANVENDELSE AF NUTRACEUTISKE SAMMENSÆTNINGER I DYREFODER.**

(56) Fremdragne publikationer:
EP-A1- 0 567 352
GB-A- 1 466 003
US-A1- 2003 114 435
US-A1- 2005 064 018
US-B1- 6 322 807
BROULIK P D ET AL: "Effects of triiodothyronine and estrogen administration on bone mass, mineral content and bone strength in male rats" HORMONE AND METABOLIC RESEARCH, THIEME-STRATTON, STUTTGART, DE, vol. 35, no. 9, September 2003 (2003-09), pages 527-531, XP008080718 ISSN: 0018-5043
ANONYMOUS: "Opinion of the Scientific Committee on Animal Nutrition on the use of benzoic acid in feedingstuffs for pigs for fattening" INTERNET CITATION, [Online] 2002, XP002440105 Retrieved from the Internet: URL:http://ec.europa.eu/food/fs/sc/scan/ou t100_en.pdf> [retrieved on 2007-06-29]

DESCRIPTION

[0001] The present invention relates to a novel use of a nutraceutical composition for animals, especially pigs, comprising as active ingredient benzoic acid or derivatives thereof.

[0002] Specific examples of derivatives of benzoic acid which can give rise to benzoic acid in vivo are alkali metal-alkaline earth metal- and ammonium benzoates.

[0003] The term "nutraceutical" as used herein denotes an usefulness in both the nutritional and pharmaceutical field of application. Thus, the nutraceutical compositions can find use as a complete animal feed (diet), as supplement to animal feed, and as pharmaceutical formulations for enteral or parenteral application which may be solid formulations, or liquid formulations.

[0004] It is known from EP-A-0 683 985 that animal feed compositions comprising benzoic acid or salts thereof can be used to minimize the emission of odoriferous ammonia from organic wastes, especially animal excrements and manure. It is further known that the addition of benzoic acid to the diet of weaner piglets improves the zootechnical performance of the animals.

[0005] It is also known from US2003/0114435 that some benzoic acid derivatives are useful for the prevention and/or treatment of bone diseases such as osteoporosis.

[0006] It is finally known form Broulik et al. (Hormone and Metabolic Research, vol. 35, No. 9, p. 527-531, 2003) that estradiol benzoate is useful for increasing bone resistance and mineralization in rats.

[0007] It has now been found surprisingly that in addition to the above function, benzoic acid and salts thereof have the advantage of being able to improve bone strength in animals, more precisely being able to increase bone resistance and mineralization, which has a significant effect to the general health status of the animal.

[0008] The term "bone resistance" as used herein denotes the maximal force in measured in Newton (N) necessary to break the bone determined by a compression machine.

[0009] The term "bone mineralization" as used herein denotes the percentage of ash after incineration of the bone compared to the dried bone matter (DM). Dry matter is defined as the weight of the bone sample after 24 hour in a drying oven compared to the initial weight of the sample.

[0010] Accordingly, the present invention relates to the use of benzoic acid or a derivative of benzoic acid capable of giving rise to free benzoic acid in vivo in the manufacture of a neutraceutical composition for increasing bone resistance and mineralization in animals,

especially pigs.

[0011] Benzoic acid or a derivative thereof capable of giving rise to benzoic acid in vivo is administrated to the animals as a component of a nutraceutical composition which is conventionally fed to animals. Thus, benzoic acid or said derivative may be suitably administered to the animals as a component of the animal feed or in their drinking water.

[0012] The amount of benzoic acid or a derivative thereof capable of giving rise to benzoic acid in vivo f administered to the animal is in the range from 0.001 - 5% based on the total weight of each feed fed to the animal. This amount may, however, be higher if the function of benzoic acid or said derivative is also to control the pH of the animal excreta fed on such a diet in order to suppress the emission of ammonia from the excreta. Such higher amounts are suitably limited to a maximum of about 10% based on the total animal feed composition.

[0013] The benzoic acid or derivative thereof capable of giving rise to benzoic acid in vivo is used in an amount sufficient to provide a daily dosage of 200 mg per kg body weight to about 600 mg per kg body weight of the subject to which it is to be administered.

[0014] The use according to the invention achieves in the animal the following bone strength parameters: a bone resistance of at least 500 N measured with metacarpal bone and/or a bone mineralization of at least 60% of ash compared to the dried bone matter (DM). In a preferred embodiment the feed comprises benzoic acid in an amount sufficient to achieve in the animal the following bone strength parameters: a bone resistance of at least 550 N, preferably 590 to 650 N measured with metacarpal bone.

[0015] A typical formulation for an animal feed composition is shown in Table 1 below in which all the amounts shown in % by weight were fed to pigs:

Table 1

Ingredients	**%**
Meat meal (58% Crude protein)	3.20
Molasses	5.00
Wheat	5.90
Soybean meal (45% crude protein)	15.10
Tapioca (66% starch)	35.50
Wheat middlings	15.00
Animal fat	3.30
Limestone	0.74
Lysine hydrochloride (98%)	0.06
Vitamin premix	0.50
Trace minerals	0.50
Sunflower meal	12.20

Ingredients	%
Amonium chloride	2.00
Amonium benzoate	1.00

[0016] Thus benzoic acid or a derivative thereof capable of giving rise to benzoic acid in vivo may be used in combination with conventional ingredients present in an animal feed composition (diet) such as calcium carbonates, elelctrolytes such as ammonium chloride, proteins such as soya bean meal, wheat, starch, sunflower meal, corn, meat and bone meal, amino acids, animal fat, vitamins and trace minerals.

[0017] In such a composition, the ratio of the electrolyte to the benzoic acid or said derivative is suitably in the range from 0.5 : 1 to 5 : 1 w/w, preferably from 1.5 : 1 to 3 . 1 w/w.

[0018] Benzoic acid or a derivative thereof capable of giving rise to benzoic acid in vivo is particularly effective to improve the digestibility of proteins and the assimilation of amino acids and nitrogen in animals such as poultry, pigs or cattle, especially pigs.

[0019] The present invention is further illustrated with reference to the following Example, which shows the effects of benzoic acid on the ileal apparent digestibility of nitrogen, energy and amino acids in the ileo-rectal anastomosed piglets Sixty-four Large-White x Landrace x Piétrain weaner piglets having an initial body weight of 8.5±1.11 kg were used. The animals were allocated into two equal groups (A1 and B1) and housed in cages in sub-groups of 6 animals each in an environmentally controlled room. Each cage had a plastic-coated welded wire floor and was equipped with 2 water nipples and 2 stainless-steel feeders. Room temperature was initially 27°C and was lowered weekly by about 2°C until 21-22°C. Environment humidity percentage throughout the experiment was 50 %. The animals were fed the basal diet (group A1) or the diet A with addition of 0.5 % of benzoic acid (VevoVitall®, DSM Nutritional Products ltd, Switzerland) (group B1). Both diets were in mash form. The composition of the diets and the concentration of benzoic acid in the diet B1 are presented in the table 1. The basal diet A1 was formulated to meet the animals' requirements according to Henry et al. (1989, L'alimentation des animaux domestiques - porc, lapin, volaille, 2nd edition, INRA, Paris) and NRC (1998), which publication is herein incorporated as reference.

[0020] After the post-weaning observation the animals of each group were divided into 2 equal groups of 16 animals each and were housed in floor-pen cages in sub-groups of 4 animals each in an environmentally controlled room for an observation period of 91 days. Each pen had a plastic-coated welded wire floor and was equipped with two water nipples and four stainless-steel individualised feeders. Room temperature was 21-22° C and humidity percentage was 50 %. Pigs ingesting during the post-weaning phase the diet without benzoic acid were fed the basal diet (group A2) or the A2 diet supplemented with 1 % of benzoic acid (group B2). Pigs fed during the post-weaning phase the diet supplemented with 0.5 % of benzoic acid received the A2 diet supplemented with 0.5 % of benzoic acid (group C2) or the

A2 diet supplemented with 1 % of benzoic acid (group D2). The composition of the diets and the concentration of benzoic acid in the diets B2, C2 and D2 are presented in the table 2. All the diets were given in a mash form. The basal diet A2 was formulated to meet the animals' requirements according to Henry et al. (1989) and NRC (1998). The determination of the concentration of benzoic acid was performed according to a standard method.

[0021] At the end of the observation all the animals were slaughtered after electronarcosis for the determination of the bone resistance and mineralization. The collected bones were the right internal and external metacarpal. Samples were prepared from each of the collected bones immediately after slaughter. After careful removal of the soft tissue, a diaphysis section was obtained by sawing each bone. The obtained sections of about 3.5-cm long were immediately subjected to compression in order to determine the force in Newton necessary to break them (maximal-breaking force at the fracture point). The measurements were performed with a LR10K compression machine, using a XLC/10K/A1 force captor and a compression device TH23-196/AL (Lloyd Instruments, Fareham, UK). The broken bones were then dried in a drying oven at 105°C during 24 hours and the dry matter (DM) was used for the determination of ash content, which was measured after 48h incineration at 550°C in a muffle furnace.

[0022] Statistical treatment of the results involved the calculation of the mean and of the standard deviation as well as a two-factor hierarchical analysis of variance. The mathematical model was:

$$Yijk = \mu+Ai+Bij+Zijk,$$

where μ is the mean, Ai is the diet effect, Bij is the combined effect of the diet and animal or pen and Zijk is the residual term. The analysis of variance was followed by a Duncan multiple range test when a significant Ai effect without Bij effect was observed (Statistical methods, 8th edition, Iowa University Press, Ames, Snedecor and Cochran, 1989). These calculations were performed using the Statistical Analysis System (SAS, 1990).

[0023] The observed concentration of benzoic acid in the supplemented feed used in both phases of the experiment was in very good agreement with the programmed inclusion levels (tables 1 and 2).

[0024] The animals did not present any symptoms of illness during the experiment. Furthermore, no natural mortality was observed and thus all the initial piglets were present during all the duration of the experiment.

[0025] The bone resistance was significantly increased in all the supplemented groups (table 3). The mean resistance of the bones of the supplemented animals, groups B2, C2 and D2 represented respectively 128, 127 and 122 % of that of the controls. The ash content of the bones of the pigs ingesting supplements of benzoic acid was also increased (table 6). The ash content was significantly higher in the animals of the groups B2 and D2 than in the controls. That of the animals of the group C2 was numerically higher ($P < 0.07$) than that of the controls.

[0026] These results clearly show that the long-term ingestion of benzoic acid by swine was followed by a significant increase of the bone resistance and mineralization.

Table 1: Composition and characteristics of the experimental diets used during the post-weaning phase

Ingredients (%)	A1	B1
Soybean meal	7.4	
Wheat	15	
Barley	29.4	
Potato concentrate	8	
Maize	10	
Wheat bran	1.6	
Oatmeal	10	
Beet pulp	5.5	
Treacle	3.5	
Soya oil	2.7	
Minerals, vitamins and synthetic amino acids	6.9	
Digestible energy MJ/kg	13.9	
Crude protein (%)	17.7	
Crude fat (%)	4.9	
Lysine (%)	1.20	
Methionine+Cystine (%)	0.75	
Phosphorus (%)	0.60	
Calcium (%)	0.75	
Benzoic acid %	-	0.5
Benzoic acid in feed %	-	0.49±0.02(1)
Benzoic acid % of the target	-	98
(1) - Mean±standard deviation of 4 measurements.		

Table 2: Composition and characteristics of the experimental diets used during the growing-fattening phase

Ingredients (%)	A2	B2	C2	D2
Maize	53			
Soybean meal	18.2			
Barley	13			
Oat meal	6			
Wheat bran	5.4			
Soya oil	1			

Ingredients (%)	A2	B2	C2	D2
Minerals, vitamins and synthetic aa	3.4			
Digestible energy - MJ / kg	13.31			
Crude protein - N x 6.25	15.5			
Lysine - %	0.96			
Methionine + cystine (%)	0.54			
Phosphorus (%)	0.41			
Calcium (%)	0.66			
Benzoic acid %	-	1	0.5	1
Benzoic acid in feed %	-	0.99± 0.05(1)	0.51± 0.02(1)	0.94± 0.04(1)
Benzoic acid % of the target	-	99	102	94
(1) - Mean±standard deviation of 4 measurements.				

Table 3: Effects of the addition to the diet comprising benzoic acid on the bone resistance and mineralization of the growing-fattening pig.

A2 - 0 % of benzoic acid during 32 days and 0 % of benzoic acid during 91 days,				
B2 - 0 % of benzoic acid during 32 days and 1 % of benzoic acid during 91 days,				
C2 - 0.5 % of benzoic acid during 32 days and 0.5 % of benzoic acid during 91 days and				
D2 - 0.5 % of benzoic acid during 32 days and 1 % of benzoic acid during 91 days				
Variable	A2	B2	C2	D2
Resistance (Newton at the breaking point)				
Right metacarpals	490±104$^{(1)ac}$ (100)	625±163$^{(2)bd}$ (128)	621±172$^{(2)d}$ (127)	597±192$^{(2)d}$ (122)
Mineralization (% of ash in DM)				
Right metacarpals	59.08±1.40$^{(1)d}$ (100)	60.26±1.31$^{(1)d}$ (102)	60.06±1.33$^{(1)cd*}$ (102)	60.14±1.09$^{(1)d}$ (102)
Animals: growing pigs of an initial body weight of 21.7±2.17 kg; Diet based on: maize, barley and soybean meal; (1) - Mean±standard deviation of 32 measurements; a, b, c - Mean values within a row with unlike superscript letters were significantly different: a - b P < 0.01, c-dP<0.05; **-P<0.07.				

REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Patent documents cited in the description

- EP0683985A [0004]
- US20030114435A [0005]

Non-patent literature cited in the description

- **BROULIK et al.**Hormone and Metabolic Research, 2003, vol. 35, 9527-531 [0006]
- **HENRY et al.**L'alimentation des animaux domestiques - porc, lapin, volaille, 1989, [0019]
- **AMESSNEDECORCOCHRAN**Statistical methodsIowa University Press19890000 [0022]

Patentkrav

1. Anvendelse af benzoesyre eller alkalimetal- eller jordalkalimetal- eller ammoniumbenzoater til fremstilling af en nutraceutisk sammensætning til at øge knogleresistens og -mineralisering hos dyr, hvor benzoesyren eller alkalimetal- eller jordalkalimetal- eller ammoniumbenzoaterne anvendes i en mængde, som er tilstrækkelig til at tilvejebringe en daglig dosering på fra 200 mg pr. kg kropsvægt til ca. 600 mg pr. kg kropsvægt hos det individ, hvortil den skal administreres for at opnå følgende knoglestyrkeparametre hos dyret: en knogleresistens på mindst 500 N som målt med metacarpalknogle og/eller en knoglemineralisering på mindst 60% aske i sammenligning med knogletørstoffet (dried bone matter - DM).

2. Anvendelse ifølge krav 1, hvor foderet omfatter benzoesyre i en mængde, som er tilstrækkelig til at opnå følgende knoglestyrkeparametre hos dyret: en knogleresistens på mindst 550 N, fortrinsvis 590 til 650 N, som målt med metacarpalknogle.