(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
13 April 2017 (13.04.2017)

WIPO | PCT

(10) International Publication Number
WO 2017/062301 A1

(51) International Patent Classification:
A23K 10/40 (2016.01) *A23K 10/00* (2016.01)

(21) International Application Number:
PCT/US2016/055123

(22) International Filing Date:
3 October 2016 (03.10.2016)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
PCT/US2015/054040
5 October 2015 (05.10.2015) US

(71) Applicant: **ELI LILLY AND COMPANY** [US/US]; Lilly Corporate Center, Indianapolis, Indiana 46285 (US).

(72) Inventors: **HERR, Cory T.**; c/o Eli Lilly and Company, P.O. Box 6288, Indianapolis, Indiana 46206-6288 (US). **KUBE, John Charles**; c/o Eli Lilly and Company, P.O. Box 6288, Indianapolis, Indiana 46206-6288 (US). **TEETER, Jerold Scott**; c/o Eli Lilly and Company, P.O. Box 6288, Indianapolis, Indiana 46206-6288 (US).

(74) Agent: **MCGRAW, Elizabeth A.**; Eli Lilly and Company, P.O. Box 6288, Indianapolis, Indiana 46206-6288 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:

— *with international search report (Art. 21(3))*

WO 2017/062301 A1

(54) Title: METHODS AND FORMULATIONS FOR REDUCING BOVINE EMISSIONS

(57) Abstract: The present disclosure provides methods and formulations for reducing ammonia and carbon dioxide emissions from a bovine using lubabegron, or a physiologically acceptable salt thereof. The present disclosure also provides bovine feed additives and bovine feed compositions.

METHODS AND FORMULATIONS FOR REDUCING BOVINE EMISSIONS

Ammonia is the most abundant alkaline gas in the atmosphere. In addition, it is a major component of total reactive nitrogen. Recent studies have indicated that ammonia emissions have been increasing over the last few decades on a global scale. This is a concern because ammonia plays a significant role in the formation of atmospheric particulate matter, visibility degradation and atmospheric deposition of nitrogen to sensitive ecosystems. Additionally, carbon dioxide is a greenhouse gas linked to global warming. Thus, the increase in ammonia and carbon dioxide emissions negatively influences environmental and public health. Bovines, and particularly cattle, are major emitters of ammonia and contribute significantly to carbon dioxide emissions. Ammonia is generated and emitted by bovines during their digestive process, as well as emitted from bovine wastes as they break down.

Different approaches have been used to control ammonia and carbon dioxide emissions from bovines. One set of methods for reducing ammonia and carbon dioxide emissions are dietary manipulation strategies. One such approach applied to reducing both ammonia and carbon dioxide emissions is to reduce the amount of protein fed to the bovine. However, such a lower protein approach can lead to lesser amounts or slower accumulation of desired bovine muscle. In addition to dietary manipulation strategies, many other practices have been utilized for reducing ammonia emissions, such as filtration of emissions and particles, building impermeable barriers to prevent the movement of ammonia emissions, and control strategies for feces and urine in bovine raising operations. Many of these practices are costly, inconvenient, and of limited benefit. Therefore, there exists a need for alternatives for reducing bovine ammonia and carbon dioxide emissions. Preferably, such alternatives decrease the inconvenience, drawbacks, and/or cost of one or more of the current approaches.

U.S. Patent No. 6,730,792 ('792) discloses lubabegron and salts thereof for use in treating Type II diabetes and obesity and for binding to and activating the β_3

receptor. Additionally, '792 states that in non-human, non-companion animals, the compounds of formula I described therein are useful for increasing weight gain and/or improving the feed utilization efficiency and/or increasing lean body mass and/or decreasing birth mortality rate and increasing post/natal survival rate. However, lubebagron or salts thereof was not known to reduce ammonia and/or carbon dioxide emissions from bovine.

The present invention provides a method of reducing ammonia and/or carbon dioxide emissions from a bovine in need thereof comprising orally administering to the bovine lubabegron, or a physiologically acceptable salt thereof.

Another aspect of the present disclosure provides lubabegron, or a physiologically acceptable salt thereof, for use in reducing ammonia and/or carbon dioxide emissions from a bovine.

Another aspect of the present disclosure provides lubabegron, or a physiologically acceptable salt thereof, for use in reducing ammonia and/or carbon dioxide emissions from a bovine wherein said lubabegron is to be orally administered.

Another aspect of the present disclosure provides a bovine feed additive which comprises lubabegron, or a physiologically acceptable salt thereof, and a suitable carrier therefor, wherein said additive is for the reduction of ammonia and/or carbon dioxide emissions.

Another aspect of the present disclosure provides an animal feed for reducing ammonia and/or carbon dioxide emissions from a bovine which comprises a bovine feed and lubabegron, or a physiologically acceptable salt thereof.

Lubabegron, or a physiologically acceptable salt thereof, such as lubabegron fumarate, may be made by processes known in the art. The hemifumarate salt of lubabegron is known as lubabegron fumarate (CAS Registry Number 391926-19-5). For example, the processes described in U.S. Patent No. 6,730,792 are illustrative processes that may be used to make lubabegron, or a physiologically acceptable salt thereof.

As used herein, the term "bovine" refers to an animal that is a member of the biological subfamily *Bovinae*, including but not limited to cows/cattle, bison, African buffalo, and water buffalo. In preferred embodiments, the animal is a cow. As used herein, the term "cow" is a bovine of either sex or age, and is a member of the biological genus *Bos*, including the species *Bos taurus* and *Bos indicus*. Cows in a group are commonly known as cattle. As such, the term cow includes dairy cattle, beef cattle, bulls, heifers, oxen, and steers.

As used herein, "reducing ammonia emissions" from a bovine treated with lubabegron, or a physiologically acceptable salt thereof, refers to reducing emitted ammonia gas relative to a bovine not treated with lubabegron, or a physiologically acceptable salt thereof. In some embodiments, the reduction is from about 10 to about 30% in ammonia emissions when compared to an untreated animal. In some embodiments, the reduction is from about 15 to about 25% in ammonia emissions. In some embodiments, the reduction of ammonia emissions from a bovine does not significantly negatively affect the bovine, such as, for example, lowering body weight, or decreasing meat and eating quality. In some embodiments, the reduction is per pound of live weight of the bovine. Live weight means the weight of the bovine while alive. In some embodiments, the reduction is per pound of hot carcass weight of the bovine. Hot carcass weight is the weight of a bovine carcass prior to chilling with its hide, head, gastrointestinal tract, and internal organs removed. In some embodiments, the reduction of ammonia is accompanied by an increase in hot or live carcass weight. In some embodiments, the bovine is in confinement for slaughter when administered lubabegron, or a physiologically acceptable salt thereof.

As used herein, "reducing carbon dioxide emissions" from a bovine treated with lubabegron, or a physiologically acceptable salt thereof refers to reducing emitted carbon dioxide gas relative to a bovine not treated with lubabegron, or a physiologically acceptable salt thereof. In some embodiments, the reduction is about 9% in carbon dioxide emissions when compared to an untreated animal when carbon dioxide emissions are standardized to animal live weight. In some embodiments, the

reduction is about 10% in carbon dioxide emissions when compared to an untreated animal when carbon dioxide gas emissions are standardized by animal hot carcass weight. In some embodiments, the reduction of carbon dioxide emissions from a bovine does not significantly negatively affect the bovine, such as, for example, lowering body weight, or decreasing meat and eating quality. In some embodiments, the reduction is per pound of live weight of the bovine. Live weight means the weight of the bovine while alive. In some embodiments, the reduction is per pound of hot carcass weight of the bovine. Hot carcass weight is the weight of a bovine carcass prior to chilling with its hide, head, gastrointestinal tract, and internal organs removed. In some embodiments, the reduction of carbon dioxide is accompanied by an increase in hot or live carcass weight. In some embodiments, the bovine is in confinement for slaughter when administered lubabegron, or a physiologically acceptable salt thereof. Lubabegron, or a physiologically acceptable salt thereof, can be formulated for oral administration, and such formulations include animal feeds and feed additives. In some embodiments, the administration is carried out by including lubabegron, or a physiologically acceptable salt thereof, in an animal (bovine) feed. The animal feed may be a dry feed or a liquid feed, and includes a bovine's drinking water containing lubabegron, or a physiologically acceptable salt thereof. Such animal feeds may include lubabegron, or a physiologically acceptable salt thereof, combined or admixed with suitable feedstuffs commonly employed in the feeding of bovines. Typical feedstuffs commonly employed include corn meal, corncob grits, soybean meal, alfalfa meal, rice hulls, soybean mill run, cottonseed oil meal, bone meal, ground corn, corncob meal, wheat middlings, limestone, dicalcium phosphate, sodium chloride, urea, distillers dried grain, vitamin and/or mineral mixes, cane molasses or other liquid carriers and the like. Such feedstuffs promote a uniform distribution and administration of lubabegron, or a physiologically acceptable salt thereof. In some embodiments, feedstuffs containing lubabegron, or a physiologically acceptable salt thereof, is provided to a bovine *ad libitum* (*i.e.*, "at will").

While a particular embodiment for orally administering lubabegron, or a physiologically acceptable salt thereof, is via daily feed rations, lubabegron, or a physiologically acceptable salt thereof, can be incorporated into salt blocks and mineral licks, as well as being added directly to lick tank formulations or drinking water for convenient oral consumption. Lubabegron, or a physiologically acceptable salt thereof, can also be administered orally by bolus or gavage treatment.

In some embodiments, feed additives are provided which include lubabegron, or a physiologically acceptable salt thereof, and one or more suitable carriers. The feed additive may be a dry feed additive or a liquid feed additive. The feed additives are formulated such that, when added with other materials, an animal feed is formed which will provide a desired concentration of lubabegron, or a physiologically acceptable salt thereof, in the animal feed, and/or provide the desired dose of lubabegron, or a physiologically acceptable salt thereof, to the bovine upon the bovine's consumption of the animal feed. Premixes are recognized terms in the art for certain feed additives. They may be solid or liquid. A mineral premix is a composition which is intended for formation of an animal feed and which comprises desired kinds and amounts of minerals, in particular trace minerals. A vitamin premix is a composition which is intended for formation of an animal feed and which comprises desired kinds and amounts of vitamins. Some premixes include both vitamins and minerals. As such, feed additives includes premixes such as mineral premixes, vitamin premixes, and premixes which include both vitamins and minerals.

In some embodiments, lubabegron, or a physiologically acceptable salt thereof, is administrated to the bovine up to at least 91 days prior to slaughter of the bovine. In some embodiments, lubabegron, or a physiologically acceptable salt thereof, is administrated to the bovine up to at least 14 to 56 days prior to slaughter of the bovine. In some embodiments, the period of administration ends upon the bovine's slaughter. In another embodiment, the bovine is orally administered lubabegron, or a physiologically acceptable salt thereof, in daily feed rations up to 91 days prior to slaughter.

The term "effective amount", in the context of administration, refers to the quantity of lubabegron, or a physiologically acceptable salt thereof, when administered to a bovine, which is sufficient to reduce ammonia and/or carbon dioxide emissions from the bovine, as compared to a bovine untreated with lubabegron, or a physiologically acceptable salt thereof. The term "effective amount", in the context of a feed additive, refers to the quantity of lubabegron, or a physiologically acceptable salt thereof, included in the animal feed sufficient to reduce ammonia and/or carbon dioxide emissions from a bovine, as compared to a bovine untreated with lubabegron, or a physiologically acceptable salt thereof, when the bovine consumes the animal feed.

In some embodiments, lubabegron, or the equivalent of the lubabegron free base of a physiologically acceptable salt thereof, is administered in an amount from about 1 mg/day to about 500 mg/day. In some embodiments, lubabegron, or the equivalent of the lubabegron free base of a physiologically acceptable salt thereof, is administered in an amount from about 5 mg/day to about 500 mg/day. In some embodiments, lubabegron, or the equivalent of the lubabegron free base of a physiologically acceptable salt thereof, is administered in an amount from about 10 mg/day to about 400 mg/day.

In some embodiments, the animal feed contains from about 0.5 to about 100 grams of lubabegron, or the equivalent of the lubabegron free base of a physiologically acceptable salt thereof, per ton of animal feed. In some embodiments, the animal feed contains from about 0.5 to about 50 grams of lubabegron, or the equivalent of the lubabegron free base of physiologically acceptable salt thereof, per ton of animal feed. In some embodiments, the animal feed contains from about 1 to about 25 grams of lubabegron, or the equivalent of the lubabegron free base of a physiologically acceptable salt thereof, per ton of animal feed. In some embodiments, the animal feed contains from about 1.25 to about 20 grams of lubabegron, or the equivalent of the lubabegron free base of a physiologically acceptable salt thereof, per ton of animal feed.

In some embodiments, the present disclosure includes the use or inclusion of additional active ingredients. In some embodiments, the additional active ingredients are one or more selected from the group consisting of monensin, tylosin, and melengestrol, or physiologically acceptable salts thereof.

The terms and phrases in the Example have their ordinary meaning as understood by one of ordinary skill in the art.

Example 1, Reduction of Ammonia Emissions:

Prepare lubabegron (L) as 4.5 g/lb of Type A Medicated Article. In a facility having at least eight cattle pen enclosures (CPEs), test two cycles of cattle, each cycle representing all dose (0, 1.25, 5, and 20 g/ton) and gender (steer and heifer) combinations. For the purpose of this example, a cycle refers to a group of 112 animals housed concurrently. Within each cycle, there are 2 cohorts of animals (56 animals per cohort). A cohort refers to a group of same gender animals representing each of the 4 doses. Up to 4 cycles are used to provide a total of 4 cohorts per gender.

Upon receipt of the cattle, allocate the cattle to CPEs to acclimate for 7 days. After the acclimation phase, for 91 days orally treat via feed one fourth of the cattle allocated to CPEs L 0 g/ton/day; one fourth 1.25 g/ton/day; 5 g/ton/day; and 20 g/ton/day (100% dry matter basis). Provide feed and water *ad libitum*. On day 91, collect body weight and load cattle for transport to the slaughter facility. On day 92, slaughter the cattle and evaluate the carcass. During the study, monitor and collect ammonia gas emissions data. Measure the ammonia emissions over the treatment period and normalize by body weight (BW) for the period (Days 0-7, 0-14, 0-28, 0-56, and 0-91) and hot carcass weight (HCW) (Days 0-91) (g of gas/animal; g of gas/lb of live BW; g of gas/lb of HCW). Using the process described above, the following results are achieved.

Reduction, as compared to control	g of NH_3 gas/lb of live BW (g of NH_3 gas/animal)					g of NH_3 gas/lb of HCW
Day	0-7	0-14	0-28	0-56	0-91	0-91
1.25 g/ton	5% (5%)	14% (12%)	16% (15%)	13% (11%)	11% (9%)	13%
5 g/ton	8% (7%)	17% (16%)	21% (20%)	18% (16%)	14% (12%)	16%
20 g/ton	22% (21%)	27% (27%)	26% (25%)	19% (19%)	15% (13%)	17%

Example 2, Reduction of Carbon Dioxide Emissions:

Prepare lubabegron (L) as 4.5 g/lb of Type A Medicated Article. In an appropriate facility having cattle chambers or rooms for individual animals (chambers), test ten cycles of twelve cattle each, each cycle representing all dose (0, 1.25, 5, and 20 g/ton) with a mixture of genders (steer and heifer).

Upon receipt of the cattle, allocate the cattle to chambers to acclimate for 7 days. After the acclimation phase, for 14 days orally treat via feed one fourth of the cattle allocated to chambers L 0 g/ton/day; one fourth 1.25 g/ton/day; 5 g/ton/day; and 20 g/ton/day (100% dry matter basis). Provide feed and water *ad libitum*. On day 14, collect body weight and load cattle for transport to the slaughter facility. On day 15, slaughter the cattle and evaluate the carcass. During the study, monitor and collect carbon dioxide gas emissions data. Measure the carbon dioxide emissions over the treatment period and normalize by body weight (BW) for the periods (Days 0-7, 0-14, and 7-14) and hot carcass weight (HCW) (Days 0-14) (g of gas/animal; g of gas/lb of live BW; g of gas/lb of HCW). Using the process described above, the following results are achieved.

Reduction, as compared to control	g of CO_2 gas/lb of live BW (g of CO_2 gas/animal)			g of CO_2 gas/lb of HCW	
Day	0-7	0-14	7-14	0-14	7-14
1.25 g/ton	0% (0.2%)	2% (3%)	5% (5%)	4%	6%
5 g/ton	3% (4%)	6% (7%)	9% (10%)	7%	10%
20 g/ton	4% (4%)	6% (7%)	9% (9%)	7%	10%

We Claim:

1. A method of reducing one or more gas emissions selected from the group consisting of ammonia and carbon dioxide from a bovine in need thereof comprising orally administering to said bovine lubabegron, or a physiologically acceptable salt thereof.

2. The method of claim 1, wherein the gas emission is ammonia.

3. The method of claim 1 or 2, wherein lubabegron, or a physiologically acceptable salt thereof, is the hemifumarate salt thereof.

4. The method of claim 1, 2 or 3, wherein said lubabegron, or a physiologically acceptable salt thereof, is administered in an animal feed.

5. The method of any of claims 1 to 4, wherein one or more other active ingredients are administered to said bovine, wherein said other active ingredients are one or more selected from the group consisting of monensin, tylosin, and melengestrol, or physiologically acceptable salts thereof.

6. The method of any of claims 1 to 5, wherein said bovine is a cow.

7. The method of any of claims 1 to 6, wherein said reduction is per pound of live weight of said bovine.

8. The method of any of claims 1 to 5, wherein said reduction is per pound of hot carcass weight of said bovine.

9. Lubabegron, or a physiologically acceptable salt thereof, for use in reducing ammonia emissions from a bovine wherein said lubabegron is to be orally administered.

10. A bovine feed additive comprising lubabegron, or a physiologically acceptable salt thereof, and a suitable carrier therefor, wherein the said additive reduces one or more gas emissions selected from the group consisting of ammonia and carbon dioxide.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2016/055123

A. CLASSIFICATION OF SUBJECT MATTER
INV. A23K10/40 A23K10/00
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A23K

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 practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DATABASE WPI Week 201144 Thomson Scientific, London, GB; AN 2011-F90007 XP002752309, & NL 2 002 197 C (JOZ BV) 17 May 2010 (2010-05-17) abstract ----- DATABASE WPI Week 199924 Thomson Scientific, London, GB; AN 1999-286592 XP002752310, & NL 1 006 953 C6 (INST MILIEU EN AGRITECHNIEK IMAG DLO) 8 March 1999 (1999-03-08) abstract ----- -/--	1-10 1-10

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* 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 application or patent but published on or after the international filing date

"L" document which may throw doubts 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

"T" 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 combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 January 2017	19/01/2017

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Georgopoulos, N

3

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

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DATABASE WPI Week 199436 Thomson Scientific, London, GB; AN 1994-291702 XP002752311, & NL 9 300 228 A (CABO-DLO CENT AGROBIOLOGISCH ONDERZOEK) 1 September 1994 (1994-09-01) abstract	1-10

X	US 6 730 792 B2 (EVERS BRITTA [DE] ET AL) 4 May 2004 (2004-05-04) cited in the application	10
A	* columns 57-60; column 61, line 53 - column 62, line 47; claims 1-17 *	1-9

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2016/055123

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
NL 2002197 C	17-05-2010		
NL 1006953 C6	08-03-1999	NONE	
NL 9300228 A	01-09-1994	NONE	
US 6730792 B2	04-05-2004	AU 7291701 A	30-01-2002
		BR 0112409 A	22-07-2003
		CA 2415331 A1	24-01-2002
		CN 1441800 A	10-09-2003
		CZ 20030106 A3	16-04-2003
		DK 1303509 T3	02-01-2013
		EP 1303509 A1	23-04-2003
		ES 2398097 T3	13-03-2013
		HR P20030018 A2	30-04-2003
		HU 0301329 A2	28-08-2003
		IL 152528 A	24-12-2009
		JP 4988128 B2	01-08-2012
		JP 2004504320 A	12-02-2004
		KR 20030016396 A	26-02-2003
		MX PA03000308 A	06-06-2003
		NO 20030098 A	09-01-2003
		PE 01952002 A1	12-03-2002
		PL 360098 A1	06-09-2004
		PT 1303509 E	24-01-2013
		SK 632003 A3	03-06-2003
		SV 2002000522 A	24-10-2002
		US 2003191156 A1	09-10-2003
		US 2004242633 A1	02-12-2004
		WO 0206276 A1	24-01-2002