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(54) FEED CONTAINING ENDURACIDIN

(71) We, TAKEDA YAKUHIN KOGYO KABUSHIKI KAISHA (TAKEDA CHEMICAL INDUSTRIES, LTD.) a corporation organised under the laws of Japan, of 27, Doshomachi 2-chome, Higashi-ku, Osaka, Japan, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to a method for accelerating the gain in body weight of livestock, and also to a feed composition or an additive thereto for accelerating the gain in body weight of livestock.

It has been found in recent years that antibiotics such as aureomycin, when fed to livestock, accelerate the gain in body weight of the livestock.

Although aureomycin has been known as useful for accelerating the gain in body weight of the livestock, new materials that would produce a heavier weight gain more rapidly and which would attain the purpose more efficiently relative to the feed intake at a lower cost and without undesirable side effects have still been extensively sought.

The present invention provides a method of

accelerating a heavier gain in the body weight of livestock, a feedstuff composition the feeding of which results in accelerating a heavier gain in the body weight of livestock, and an additive composition comprising enduracidin, in the form of a concentrate compounded with a carrier edible by the livestock.

These results are achieved by feeding an effective amount of enduracidin to the livestock, in association with a feedstuff or drinking water.

Enduracidin, metabolite of microorganisms has a polypeptide structure, and its physico-chemical properties are as detailed below. It is recovered from the culture broth of enduracidin producing micro-organisms, e.g. *Streptomyces fungicidicus* No. 5477 (A.T.C.C. 21013 and 21014) for example as described in British Patent No. 1,163,270.

The term "Enduracidin" as used herein should be taken as meaning the enduracidin-free base and/or its pharmaceutically acceptable acid addition salts such as the hydrochloride thereof, unless otherwise noted.

Details of physico-chemical properties of the enduracidin-free base are as follows:

1. Elementary analysis:

Enduracidin consists of C, H, N, O and Cl atoms; Enduracidin contains $3.36 \pm 0.5\%$ chlorine atom in its molecule.

2. Specific rotation:

$$[\alpha]_{D^{25}} = +85^{\circ} \pm 10^{\circ} (C=0.5, \text{ in dimethylformamide})$$

3. Absorption spectra:

$$\begin{array}{lcl} 95\% \text{ MeOH} & \left\{ \begin{array}{l} 230 \text{ m}\mu (E_{1\text{cm}}^{1\%}) = 220 \pm 15 \\ \lambda_{\text{max}} \quad \quad \quad 263 \text{ m}\mu (E_{1\text{cm}}^{1\%}) = 150 \pm 10 \end{array} \right. \\ \\ \text{N}/10 \text{ HCl} & \left\{ \begin{array}{l} 230 \text{ m}\mu (E_{1\text{cm}}^{1\%}) = 195 \pm 10 \\ \lambda_{\text{max}} \quad \quad \quad 272 \text{ m}\mu (E_{1\text{cm}}^{1\%}) = 112 \pm 10 \end{array} \right. \\ \\ \text{N}/10 \text{ NaOH} & 250 \text{ m}\mu (E_{1\text{cm}}^{1\%}) = 335 \pm 10 \\ \lambda_{\text{max}} & \end{array}$$

The significant infra-red absorption bands in microns (KBr-method):

3.05	(strong)	6.90	(middle)
3.25	(shoulder)	7.23	(middle)
3.38	(shoulder)	7.63	(middle)
5.75	(shoulder)	8.10	(broad, strong)
5.95	(shoulder)	8.51	(middle)
6.09	(strong)	9.90	(middle)
6.24	(shoulder)	10.40	(weak)
6.47	(shoulder)	11.92	(middle)
6.55	(shoulder)	12.30	(weak)
6.61	(strong)	12.75	(very weak)

4. Colour:

Colourless powder

5. Colour reaction:

reagent	result
Ninhydrin reagent	positive (pale-brownish violet)
Barton's reagent (a mixture of equal volumes of 1% aqueous ferric chloride and 1% aqueous potassium ferri-cyanide)	positive (pale-blue)
Alkaline potassium permanganate	discolouration occurs (reduction)

6. Melting point:

205°C. to 225°C. (sintering) 225°C. to 240°C. (decomposition)

7. Solubility

5 Enduracidin is readily soluble in pyridine, dimethylformamide, dilute hydrochloric acid, aqueous alkaline solution (with decomposition), soluble in aqueous methanol, aqueous ethanol, aqueous acetone and aqueous butanol, but insoluble or hardly soluble in water, absolute

ethanol, pure butanol, pure acetone.

8. Rf value (paper partition chromatography): 10

(Measured by using the ascending method on "Whatman (Trade Mark) filter paper No. 1" (W. and R. Balston Ltd., Great Britain).

Solvent	Rf value*
Acetic acid/n-Butanol/Water (1:4:5)	0.45±0.1
n-Butanol saturated with water	0.0
Pyridine/n-Butanol/water (3:4:7)	0.80±0.1

* The substance is easily adsorbed on filter paper under neutral conditions.

Enduracidin may be used in its pure state or in its crude state such as in culture broth, especially the culture medium of an enduracidin-producing microorganism, e.g. *Streptomyces fungicidicus* No. 5477, A.T.C.C. Nos. 21013 and 21014), or extracts thereof.

It is now possible by the use of enduracidin according to the present invention to obtain unexpected results in raising meat-producing livestock, the results being greater than are achieved by the use of an antibiotic such as aureomycin.

These unexpected results include an increased rate of weight gain, a high extent of weight gain and a high feed efficiency in feed conversion, for example.

The foregoing results are directly connected with an improved raising of meat-producing livestock such as chicks, layers, broilers, sows, pigs, calves, ducks, and turkeys.

The effective amount of enduracidin for accelerating gain in body weight varies with the size, kind and growth stage of the livestock, but falls usually within the range of from 0.1 to 10, preferably 0.5 to 5 milligrams per kilogram of the body weight per day.

In order to practise the method for accelerating the gain in body weight of livestock, it is convenient in practice to have an effective amount of enduracidin dispersed in the feedstuff or drinking water for the livestock.

As to the feed composition, the effective amount of enduracidin for the livestock is about 2 to 400 milligrams, preferably 5 to 50 milligrams, of enduracidin per kilogram of the feedstuff.

A suitable concentration of enduracidin to be dispersed in the livestock's drinking water is about 4 to 1000 milligrams, preferably 30 to 150 milligrams, per liter of the water.

When enduracidin is added to the feedstuff or drinking water, it is preferable that the additives should take the form of concentrates.

By selecting a proper diluent and by altering the ratio of edible carrier to enduracidin,

a feed composition of any desired concentration may be prepared.

Formulations containing enduracidin at a ratio of 1 to 40% by weight, preferably 2 to 25% by weight, are particularly convenient for use as additives to livestock feedstuffs.

Moreover, enduracidin is intimately dispersed in or admixed through a feedstuff or an edible carrier for the livestock by means of, for example, grinding, stirring, milling or tumbling.

It is to be noted, however, that the suitable concentration or suitable dose of enduracidin is to be determined in accordance with the kinds of livestock, age, body weight, and average amount of feed consumed daily, and the determination can be made by a conventional means familiar to those ordinarily skilled in the art.

In addition, the livestock's drinking water or feedstuff may also contain antibiotics such as aureomycin, Mikamycin, Oleandomycin, Penicillin, Tetracyclin, amino acids such as glutamic acid, aspartic acid, leucine, lysine, tryptophane, valine, serine, proline, glucine, alanine, isoleucine, phenylalanine, arginine, methionine, threonine, or their salts, vitamins such as Vitamin B₁, Vitamin B₂, Vitamin B₆, Vitamin B₁₂, Vitamin C, Vitamin D, biotin, folic acid, Vitamin K, Vitamin E, Vitamin P, inositol, orotic acid and α -lipoic acid.

The median lethal dose (LD₅₀) of enduracidin against acute toxicity in mice is not less than 880 milligrams per kilogram of body weight when administered intraperitoneally and not less than 66 milligrams per kilogram of body weight when administered intravenously).

The median lethal dose (LD₅₀) of enduracidin against acute toxicity in rats is not less than 300 milligrams per kilogram of body weight when administered intravenously.

For the purpose of giving those skilled in the art a better understanding of the invention, the following comparative tests and examples are now given.

TEST 1

400 broiler chickens (Goto-Hiiku No. 3),
3 days' old, each weighing about 60 grams,
were divided in equal numbers (females 50,
5 males 50) into four groups.

22.2 milligrams of enduracidin per kilogram
of the feedstuff were fed to two groups, re-
ferred to as the test groups.

22.2 milligrams of auromycin per kilo-
10 gram of the feedstuff were fed to the other

two groups, referred to as the control groups.
Each group was raised by so-called floor
feeding and *ad. libitum* feeding.

From the start to 30 days' old, the feedstuffs
(A) and (B) of broiler starter on the List of 15
Feedstuffs shown below were fed to the test
groups and the control groups, respectively,
and thereafter the feedstuffs (C) and (D) of
broiler finisher on the List were fed to the test
groups and the control groups, respectively. 20

LIST OF FEEDSTUFFS

Broiler

Ingredients	Starter (weight %)		finisher (Weight %)	
	Feedstuff (A)	Feedstuff (B)	Feedstuff (C)	Feedstuff (D)
Corn	54.85	54.85	56.65	56.65
Soybean oil meal (45%)	16.00	16.00	14.00	14.00
Wheat meal	5.00	5.00	5.00	5.00
Fish meal (60%)	10.00	10.00	8.00	8.00
Fish solubles	3.00	3.00	3.00	3.00
Alfalfa meal	3.00	3.00	3.00	3.00
Tallow (30%)	6.00	6.00	8.00	8.00
Calcium phosphate	1.60	1.60	1.60	1.60
Table salt	0.25	0.25	0.40	0.40
Lipophilic vitamin premix (containing A, D and E)	0.05	0.05	0.05	0.05
Vitamin B premix (containing B ₁ , B ₂ , B ₆ and B ₁₂)	0.15	0.15	0.15	0.15
Mineral Premix (commercially available)	—	—	0.05	0.05
2.2% Enduracidin premix (dispersed in lactose)	0.10	—	0.10	—
2.2% Chlortetracyclin (Aurofac-10) "Aurofac" is a Trade Mark.	—	0.10	—	0.10
Total	100.00	100.00	100.00	100.00

TABLE 1

Age Groups	10 days-old		20 days-old		30 days-old		40 days-old		50 days-old		60 days-old	
	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test
Survival Ratio.	100	100	100	100	100	100	99.0	100	99.0	100	99.0	99.0
Body weight (g)	146	161	354	375	635	649	926	942	140	1205	1522	1613
Coefficient* of growth (%)	100	110.3	100	105.9	100	102.2	100	101.7	100	105.7	100	105.9
Feed** Conversion ratio	2.13	2.12	1.95	1.80	2.01	1.96	2.39	2.29	2.62	2.42	2.70	2.52

* The ratio of increase in body weight to that of control.

** The ratio of weight in kilogram of feedstuff required to gain one kilogram of body weight.

The above Table 1, indicates that the test group shows a 5 to 11% and about 0.15 increase in comparison with the control group on body weight and feed conversion ratio, respectively, at the stages of 10 and 20 days' old. Furthermore, the test group shows an about 5% and 0.18 increase in comparison with the control group on the average body weight and feed conversion ratio, respectively, at the stage of 60 days' old.

TEST 2

400 broiler chickens (Goto-Hiiku No. 3) of 3 days' old each weighing about 60 grams,

were divided in equal number (females 50, males 50) into four groups.

15 milligrams of enduracidin per kilogram of the feedstuff of the same composition as used in Test 1 were fed to two groups referred to as the test groups.

15 milligram of aureomycin per kilogram of the feedstuff of the same composition as above were fed to the other two groups, referred to as the control groups.

Each group was raised by the so-called floor feeding and *ad libitum* feeding.

The result is as in Table 2.

TABLE 2

Age	10 days-old		20 days-old		30 days-old		40 days-old		50 days-old		60 days-old	
	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test
Survival ratio (%)	100	100	100	100	100	100	100	100	99	100	99	100
Body weight (g)	145	158	364	386	604	616	928	951	1163	1218	1512	1585
Coefficient of* growth (%)	100	108.9	100	106.0	100	102.0	100	102.5	100	104.7	100	104.8
Feed conversion ratio **	1.76	1.66	1.92	1.83	2.21	2.16	2.48	2.41	2.72	2.60	2.89	2.74

*, **: the same as Test 1.

From the above Table 2, the test group showed a 6 to 9% increase in comparison with the control group on the average body weight and about 0.1 increase in feed conversion ratio, respectively, at the stages of 10 and 20 days old. Furthermore, the test group showed about 4.8% and 0.13 increase in comparison with the control group on the average body weight and feed conversion ratio, respectively, at the stage of 60 days old.

EXAMPLE 1

Broiler

Ingredient	Starter (weight %)	Finisher (weight %)
Corn	54.85	56.65
Soybean oil meal (45%)	16.00	14.00
Wheat meal	5.00	5.00
Fish meal (60%)	10.00	8.00
Fish solubles	3.00	3.00
Alfalfa meal	3.00	3.00
Tallow (30%)	6.00	8.00
Calcium phosphates	1.60	1.60
Table salt	0.25	0.40
Lipophilic vitamin premix (containing A, D and E)	0.05	0.05
Vitamin B premix (containing B ₁ , B ₂ , B ₆ and B ₁₂)	0.15	0.15
Mineral premix (commercially available)	—	0.05
2.2% Enduracidin premix (dispersed in lactose)	0.10	0.10
Total	100.00	100.00

EXAMPLE 2**Chick**

Ingredients	Starter (gram)	Pre-starter (gram)
Corn	600	1000
Soybean oil meal (44%)	560	600
Wheat	600	
Alfalfa meal	60	20
Fish meal (60%)	50	100
Dried whey	50	50
Meat and bone scraps (50%)		50
Condensed fish solubles		60
Distiller's dried solubles		50
Calcium phosphate	30	20
Ground oyster shell or limestone	30	30
Salt	10	10
Manganese sulphate	0.5	0.5
Feed Flavour	0.5	1
10% Enduracidin premix (dispersed in lactose)	0.2	0.5

EXAMPLE 3**Broiler starter**

Ingredients	Gram
Corn	1050
Soybean oil meal (50%)	625
Alfalfa meal	50
Poultry by-product meal	50
Dried whey	50
Fat	90
Calcium phosphate	30
Ground oyster shell or limestone	35
Manganese sulphate	0.5
Feed Flavour	0.5
10% Enduracidin (dispersed in lactose)	0.2

EXAMPLE 4**Broiler finisher**

Ingredients	gram
Corn	700
Wheat	600
Soybean oil meal (50%)	320
Poultry by-product meal	100
Distiller's dried solubles	50
Condensed fish solubles	50
Alfalfa meal	60
Fat	50
Calcium phosphate	30
Ground oyster or limestone	30
Salt	10
Manganese sulphate	0.5
Feed Flavour	0.5
10% Enduracidin (dispersed in lactose)	0.2

EXAMPLE 5**Pig milk replacer.**

Ingredients	Starter (gram)	Pre-starter (gram)
Corn meal	30	10
Wheat meal	28	23
Soybean powder (treated at 120°C.)	25	20
Fish meal	8	5
Molasses	4.5	
10% Enduracidin premix (dispersed in lactose)	0.05	0.05
NaCl	0.3	0.3
Precipitated calcium carbonate	0.4	0.45
Calcium secondary phosphate	0.6	0.7
2.2% Aureomycin premix (dispersed in soybean cake)	0.2	0.3
Pepsin	0.1	0.1
Spice for pig	0.05	0.05
Vitamin B ₁₂ (2.2%)	0.05	0.1
Sodium cyclohexylsulphamate	0.09	0.1
Non-fat milk powder	—	25
Sugar	—	7

EXAMPLE 6**Pig pre-starter**

Ingredients	gram
Ground yellow corn	435
Sucrose	200
Dried skimmed milk	600
Fish meal (60%)	100
Soybean oil meal (44%)	400
Dried whey	100
Condensed fish solubles	40
Fat	80
Calcium phosphate	15
Ground oyster shell or limestone	10
Salt, iodised	10
Manganese sulphate	0.25
10% Enduracidin premix (dispersed in lactose)	0.5
Sodium glutamate	1

EXAMPLE 7**Sow feed**

Ingredients	gram
Corn	1340
Wheat middlings	300
Soybean oil meal (44%)	200
Tankage	60
Alfalfa meal	60
Calcium phosphate	10
Ground oyster shell or limestone	20
Salt	10
Manganese sulphate	0.25
10% Enduracidin premix (dispersed in lactose)	0.65

EXAMPLE 8**Calf starter**

Ingredients	gram
Corn	560
Wheat Middlings	400
Soybean oil meal (44%)	400
Linseed meal	300
Distiller's dried solubles	50
Alfalfa meal	100
Condensed fish solubles	50
Molasses	100
Ground oyster shell or limestone	30
Salt	10
Manganese sulphate	0.25
Sodium glutamate	0.5
10% Enduracidin premix (dispersed in lactose)	0.3

EXAMPLE 9

Duck grower

Ingredients	gram
Corn	500
Wheat	764
Soybean oil meal (44%)	60
Alfalfa meal	50
Wheat middling	200
Fish meal (60%)	50
Meat and bone scraps (50%)	100
Oats, pulverised	10
Delactosed whey	10
Ground oyster shell or limestone	40
Salt	5.5
Manganese sulphate shell or limestone	0.5
10% Enduracidin premix (dispersed in lactose)	0.5
Sodium glutamate	2

WHAT WE CLAIM IS:—

1. A method for accelerating the gain in body weight of livestock, which comprises feeding to the livestock from 0.1 to 10 milligrams per kilogram of the body weight per day of enduracidin. 25
2. A method according to claim 1, wherein the enduracidin is in the form of an acid addition salt. 30
3. A method according to claim 2, wherein the acid addition salt is the hydrochloride. 35
4. A feed composition for enhancing the gain in body weight of livestock, which comprises a livestock feedstuff having dispersed therein 5 to 50 milligrams of enduracidin per kilogram of the feedstuff. 40
5. A feed composition according to claim 4, wherein the enduracidin is in the form of an acid addition salt.
6. A feed composition according to claim 5, wherein the acid addition salt is the hydrochloride.
7. A composition for accelerating the gain in body weight of livestock, which comprises, as an effective ingredient, 1 to 40 per cent by weight of enduracidin, in admixture with a carrier edible by the livestock.
8. A composition according to claim 7, wherein the enduracidin is in the form of an acid addition salt.
9. A composition according to claim 8, wherein the acid addition salt is the hydrochloride.
10. A method according to claim 1 substantially as herein described with reference to any of the specific examples.
11. A composition according to claim 4 or 7 substantially as herein described with reference to any of the specific examples.

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EXAMPLE 9

Duck grower	
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Corn	500
Wheat	764
Soybean oil meal (44%)	60
Alfalfa meal	50
Wheat middling	200
Fish meal (60%)	50
Meat and bone scraps (50%)	100
Oats, pulverised	10
Delactosed whey	10
Ground oyster shell or limestone	40
Salt	5.5
Manganese sulphate shell or limestone	0.5
10% Enduracidin premix (dispersed in lactose)	0.5
Sodium glutamate	2

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2. A method according to claim 1, wherein the enduracidin is in the form of an acid addition salt.
3. A method according to claim 2, wherein the acid addition salt is the hydrochloride.
4. A feed composition for enhancing the gain in body weight of livestock, which comprises a livestock feedstuff having dispersed therein 5 to 50 milligrams of enduracidin per kilogram of the feedstuff.
5. A feed composition according to claim 4, wherein the enduracidin is in the form of an acid addition salt.
6. A feed composition according to claim 5, wherein the acid addition salt is the hydrochloride.
7. A composition for accelerating the gain in

body weight of livestock, which comprises, as an effective ingredient, 1 to 40 per cent by weight of enduracidin, in admixture with a carrier edible by the livestock.

8. A composition according to claim 7, wherein the enduracidin is in the form of an acid addition salt.

9. A composition according to claim 8, wherein the acid addition salt is the hydrochloride.

10. A method according to claim 1 substantially as herein described with reference to any of the specific examples.

11. A composition according to claim 4 or 7 substantially as herein described with reference to any of the specific examples.

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