European Food Safety Authority

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SCIENTIFIC OPINION

Safety and efficacy of Miya-Gold®S (*Clostridium butyricum*) as feed additive for chickens for fattening[1]

Scientific Opinion of the Panel on Additives and Products or Substances used in Animal Feed

(Question No EFSA-Q-2008-303)

Adopted on 2 April 2009

PANEL MEMBERS*

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SUMMARY

Following a request from the European Commission, the European Food Safety Authority (EFSA) was asked to deliver a scientific opinion on the safety and efficacy of the product Miya-Gold®S as a feed additive for chickens for fattening. This product has not previously been authorised in the Community.

The active agent of Miya-Gold®S are viable spores of *Clostridium butyricum*. The applicant is seeking for authorisation of the product Miya-Gold®S as a feed additive for chickens for fattening under the category: zootechnical additive (functional group: gut flora stabilisers) at a dose of 5 x 10^8 CFU kg^{-1} of complete feedingstuffs.

Four studies were provided to support the efficacy of Miya-Gold®S at the recommended dose. A significant improvement in feed to gain ratio was observed in all four studies. This was further supported by a meta-analysis which indicated a magnitude of response of 2.3 %. Therefore, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) considers that evidence of the efficacy of Miya-Gold®S has been provided at the recommended dose.

The compatibility of Miya-Gold®S with the coccidiostats monensin, diclazuril, maduramycin, robenidine, narasin, narasin/nicarbazin, semduramycin and decoquinate at the maximum authorised concentrations and with formic acid (0.5 %) has been demonstrated. Compatibility

[1] For citation purposes: Scientific Opinion of the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) on a request from the European Commission on the safety and efficacy of the product Miya-Gold®S (*Clostridium butyricum*) as feed additive for chickens for fattening. *The EFSA Journal* (2009) 1039, 1-16

* One member of the Panel did not participate in the discussion on the subject referred to above because of possible conflicts of interest.

with lasalocid sodium and salinomycin sodium has been observed only at concentrations lower than the maximum authorised levels.

As no adverse response was seen when Miya-Gold®S was included in diets at 100 times the recommended dose, the FEEDAP Panel concludes that the product is safe for chickens for fattening at the recommended dose.

The FEEDAP Panel considers that the use of Miya-Gold®S would not pose a risk for the consumer. The strain *C. butyricum* used in the product was demonstrated to lack the genetic determinants of known clostridial toxins and not to harbour acquired antibiotic resistances. In addition, toxicological studies did not raise concerns for consumer safety.

The concerns for the safety of the users are limited to possible sensitisation with particular emphasis on respiratory sensitisation since a significant proportion of the product is of respirable particle size.

The use as feed additive of *C. butyricum,* a naturally occurring soil micro-organism, is not considered to pose a risk to the environment.

Key words: zootechnical additive, gut flora stabilisers, *Clostridium butyricum*, Miya-Gold®S, micro-organism, chickens for fattening, efficacy, safety, compatibility, coccidiostats, formic acid

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BACKGROUND

Regulation (EC) No 1831/2003[2] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 4(1) of that Regulation lays down that any person seeking authorisation for a feed additive or for a new use of a feed additive shall submit an application in accordance with Article 7.

The European Commission received a request from the company PenTec Consulting[3] for authorisation of the product Miya-Gold®S, *Clostridium butyricum* MIYAIRI 588, to be used as a feed additive for chickens for fattening (category: zootechnical additive; functional group: gut flora stabiliser) under the conditions mentioned in Table 1.

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 4(1) (authorisation of a feed additive or new use of a feed additive). EFSA received directly from the applicant the technical dossier in support of this application.[4] According to Article 8 of that Regulation, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. The particulars and documents in support of the application were considered valid by EFSA as of 11 June 2008.

The additive Miya-Gold®S is a microbiological feed additive containing viable spores of *Clostridium butyricum* MIYAIRI 588. This product has not been previously authorised in the Community.

TERMS OF REFERENCE

According to Article 8 of Regulation (EC) No 1831/2003, EFSA shall determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the efficacy and the safety for the target animal(s), user and consumer and the environment of the product Miya-Gold®S, *Clostridium butyricum* MIYAIRI 588, when used under the conditions described in Table 1.

ACKNOWLEDGEMENTS

The European Food Safety Authority wishes to thank the members of the Working Group on Micro-organisms for the preparation of this opinion.

[2] OJ L 268, 18.10.2003, p.29
[3] PenTec Consulting, s.l., Passeing del Roser 135, Mirasol, ES 08195 Sant Cugat del Vallès, Barcelona, Spain
[4] Dossier reference: FAD-2008-0012

Table 1. **Register entry as proposed by the applicant**

Additive	Miya-Gold®S
Registration number/EC No/No (if appropriate)	Pending
Category(ies) of additive	Zootechnical
Functional group(s) of additive	Gut flora

Description			
Composition, description	**Chemical formula**	**Purity criteria**	**Method of analysis**
Preparation of *Clostridium butyricum* MIYAIRI 588 5×10^8 cfu/g	Not applicable	Complies with EU law in relation to microbial quality, heavy metals, toxins and undesirable substances	Standard microbial counting technique

Trade name	Miya-Gold®S
Name of the holder of authorisation	Miyarisan Pharmaceutical Co.Ltd. represented in the Community by Mitsui & Co. Deutschland GMBH (first placing Miya-Gold®S on the EU market)

Conditions of use				
Species or category of animal	**Maximum Age**	**Minimum content**	**Maximum content**	**Withdrawal period**
		CFU kg^{-1} of complete feedingstuffs		
Chickens for fattening	-	5×10^8 CFU	5×10^8 CFU	Not applicable

Other provisions and additional requirements for the labelling	
Specific conditions or restrictions for use	The use is permitted in feed containing the permitted coccidiostats: decoquinate, diclazuril, halofuginone, lasalocid, monensin & salinomycin
Specific conditions or restrictions for handling	R42, potential respiratory sensitizer Breathing protection during handling and safety glasses
Post-market monitoring	Post-marketing monitoring will be carried out jointly by Miyarisan Pharmaceutical Co.Ltd. and their EU distributor (Mitsui & Co. Deutschland GMBH) in compliance with EU Regulations concerning Feed Hygiene and Feed and

	Food Controls, namely HACCP and Traceability, routine postmarketing sampling and analysis, and formal monitoring of customer feedback through product or service complaints.
Specific conditions for use in complementary feedingstuffs	Dosage used should supply 5 x 10^8 CFU/kg final complete feedingstuff

Maximum Residue Limit (MRL)			
Marker residue	**Species or category of animal**	**Target tissue(s) or food products**	**Maximum content in tissues**
-	-	-	-

ASSESSMENT

1. Introduction

Miya-Gold®S is a zootechnical additive composed of viable spores of *Clostridium butyricum* Miyairi 588 (CBM 588).

The active agent CBM 588 has been authorised in Japan and other Asian countries as a pharmaceutical and nutraceutical product, food supplement, veterinary medicine and as a feed additive for several animal species.

The product has never been authorised in the Community. The applicant is seeking for authorisation of the product Miya-Gold®S, *Clostridium butyricum* MIYAIRI 588, as a feed additive for chickens for fattening under the category: zootechnical additive (functional group: gut flora stabilisers) at a dose of 5 x 10^8 CFU kg^{-1} of complete feedingstuffs.

2. Characterisation

2.1. Characterisation of active agent

The active agent of Miya-Gold®S are viable spores of *Clostridium butyricum* MIYAIRI 588 (CBM 588). This strain is deposited at the Fermentation Research Institute, Agency of Industrial Science and Technology, Japan,[5] but not in a European culture collection.

Biochemical and physiological characteristics of the strain *Clostridium butyricum* MIYAIRI 588 have been described and the strain has been characterised by means of biochemical and genetic techniques, such as the partial sequence of the *rrn* operon. Phage typing and RAPD PCR analysis were used to assess the identity of the production strain by analysing isolates from production cultures.[6]

The absence of neurotoxin production was assessed by PCR assay and Southern blot hybridisation for type E botulinum toxin gene, the most frequently encountered neurotoxin in *C. butyricum*.[7] Moreover, a second study demonstrated the absence of genes coding for botulinum neurotoxin A, B, F and of genes encoding for the non-toxic non-haemagglutinin (NTNH) in *C. butyricum* CBM 588.[8] A third PCR study demonstrated the absence in *C. butyricum* CBM 588 of genes encoding for *C. perfringens* toxins (α, β, ε and ι).[9]

C. butyricum CBM 588 contains a single plasmid of 6.5 kb. The nucleotide sequence of this plasmid has been analysed and none of the nine putative open reading frames codes for a known virulence factor of clostridia.[10]

The production strain has not been genetically modified.

2.2. Antibiotic resistance

The susceptibility of the production strain to the antibiotics recommended by the FEEDAP Panel in its Technical guidance on the update of the criteria used in the assessment of bacterial resistance to antibiotics of human or veterinary importance (EFSA, 2008) was tested by a

[5] Technical dossier/Annex 2.2.2.1
[6] Tecnical dossier/Annex 2.2.3
[7] Technical dossier/Annex 2.2.5
[8] Supplementary dossier December 2008/Annex SD 2.2.5.2
[9] Supplementary dossier December 2008/Annex SD 2.2.5.3
[10] Supplementary dossier December 2008/Annex SD 2.2.5.1

dilution method. The minimum inhibitory concentrations (MICs) of *C. butyricum* CBM 588 were lower than the EFSA breakpoints.[11]

2.3. Production process

In the manufacturing process, the strain, after control for purity and identity, is inoculated in a large scale fermenter under anaerobic conditions in culture media composed of corn starch, glucose, amino acid solutions and minerals. The spores of CBM 588 are harvested by centrifugation, washed, resuspended in a mixture of lactose (9 %), corn starch (60 %) and water, mixed to ensure homogeneity, and then spray dried.

2.4. The nature of the final product

The spore powder, after the spray dry process, is diluted with killed Brewers' Yeast (20 %), glucose, corn starch and aluminium silicate or zeolite as an anti-caking agent. The final concentration of the active agent in Miya-Gold®S is 5 x 10^8 CFU g^{-1}.

Prior to release, production batches of Miya-Gold®S are routinely screened for microbial purity (total plate count, absence of *Enterobacteriaceae, Staphylococcus aureus, Pseudomonas aeruginosa*, yeasts and moulds), mycotoxins (Aflatoxins B1, B2, G1, G2, Zearalenone, Ochratoxin, Vomitoxin and Fumonisin B1 and B2) heavy metals and arsenic.[12] Compliance with the limits was confirmed in three batches of the product.

Miya-Gold®S has a bimodal particle size distribution. One fraction consists of particles of around 10 µm in diameter, with larger agglomerates of particles measuring 100-200 µm in diameter. On a percent basis, 60 % particles are ≤ 100 µm in diameter, 40 % of particles are ≤ 25 µm, and around 10 % particles show a diameter of approximately 10 µm. The dusting potential was shown to be approximately 2.5 %.[13]

2.5. Stability and homogeneity

The stability of Miya-Gold®S during storage has been studied using three different batches stored for 24 months under ambient conditions. During this time, the initial spore counts slightly decreased, remaining however within the level guaranteed by the manufacturer. Experiments at 40 °C confirmed the stability of this product over a six-month period.

Miya-Gold®S is stable in premixtures containing vitamins and trace elements at 20-25ºC, 40-60 % RH over a period of six months. Data on losses during pelleting apparently showed losses of viability of approximately 50 %. The product is stable in mash and pelleted feed for a period of at least five months.[14]

The homogeneity of Miya-Gold®S was evaluated in vitamin and mineral premixtures and mash and pelleted feed when added at 1 x 10^{10} and 5 x 10^8 CFU kg^{-1}, respectively. Ten samples were taken from a single batch and analysed for count of *C. butyricum.* Microbiological analysis indicated that the product was homogeneously dispersed in both premixtures and feed (CV SD/mean: 1.08 %).[15]

[11] Technical dossier/Annex 2.2.6.2 and Supplementary dossier December 2008/Annex SD 2.2.6.2
[12] Tecnical dossier/Annex 2.1.4
[13] Technical dossier/Annex 2-1-5
[14] Tecnical dossier/Annex 2.3.1 and Supplementary dossier December 2008/Annex SD 2.3.1
[15] Tecnical dossier/Annex 2.3.2

2.6. Conditions of use

The applicant is seeking authorisation for Miya-Gold®S in chickens for fattening at a dose of 5 x 10^8 CFU kg^{-1} complete feedingstuff, with the added specific condition that it may be used in feed containing the permitted coccidiostats decoquinate, diclazuril, halofuginone, lasalocid, monensin and salinomycin.

2.7. Evaluation of the analytical methods by the Community Reference Laboratory (CRL)

EFSA has verified the CRL report as it relates to the methods used for the control of the active agent in animal feed. The Executive Summary of the CRL report can be found in the Appendix.

3. Efficacy

Four trials with Miya-Gold®S at different doses including the intended dose of 5 x 10^8 CFU kg^{-1} of complete feedingstuffs were provided by the applicant. They were carried out in the same European country but in two different locations. Male Ross 308 chickens were used, except for study 1 where also females were included, and birds were divided in cages per sex. Feeds were typical broiler feeds and were fed in mash form, *ad libitum*, except for the second study in which the feed was in pelleted form. Birds were weighed (pen weight) at the start of the trial and after 21 and 42 days (or 40 days in the case of study 2). Feed intake/pen, observations of health and mortalities/culls were recorded. From these data, body weight, average daily weight gain, feed to gain ratio and percentage mortality were calculated. Doses were confirmed by microbiological analyses.

Table 2. **Summary of performance data of chickens for fattening receiving Miya-Gold®S**

	Animals (replicates × birds per treatment)	Miya-Gold® S (CFU kg^{-1} feed)	Final body weight (kg)	Average daily gain (g day^{-1})	Feed/gain (kg kg^{-1})
Trial 1[16]	384 (24 × 4)	0	1.80	41.8	1.69[a]
		5 x 10^8	1.80	41.7	1.62[b]
		5 x 10^9	1.76	40.7	1.70[a]
		5 x 10^{10}	1.77	41.1	1.67[ab]
Trial 2[17]	1536 (12 × 64)	0	2.53	62.3	1.74[a]
		5 x 10^8	2.56	62.8	1.71[b]
Trial 3[18]	1760 (20 × 22)	0	2.60	60.9	1.77[a]
		5 x 10^7	2.56	60.1	1.76[ab]
		2.5 x 10^8	2.58	60.4	1.77[ab]
		5 x 10^8	2.60	60.9	1.74[b]
Trial 4[19]	2112 (32 ×22)	0	2.34	54.8	1.79[a]
		2.5 x 10^8	2.34	54.8	1.73[b]
		5 x 10^8	2.35	55.1	1.73[b]

[a, b]: Means with different superscripts in a column within a given trial are significantly different ($P < 0.05$)

[16] Technical dossier/Annex 4-1-1
[17] Technical dossier/Annex 3-2-1
[18] Technical dossier/Annex 3-2-2
[19] Technical dossier/Annex 3-2-3

In all four experiments, supplementation with Miya-Gold®S at a dose of 5 x 10^8 CFU kg^{-1} resulted in a significant improvement of feed conversion ratio ($P < 0.05$). No other significant differences in the measurements were observed.

Meta-analysis

Data from the fours trials were tested for homogeneity and pooled to enable a statistical meta-analysis, where $P \leq 0.05$ was considered significant.[20] Only the data corresponding to the control group and the treatment with the recommended dose (5 x 10^8 CFU kg^{-1} feed) were considered. Measurements included body weight, daily weight gain, feed intake, feed conversion ratio and mortality.

The results of the meta-analysis indicate a significant improvement in feed to gain ratio (2.3 %) and a significant reduction in feed intake (1.8 %). No other significant differences in mortality between the control and the treated groups were observed.

3.1. Compatibility with coccidiostats and formic acid

The compatibility of Miya-Gold®S with decoquinate, halofuginone, lasalocid, and salinomycin has been assessed in an *in vivo* study.[21]

Five chickens were housed per cage and fed *ad libitum* with one of the allocated feeds for ten days. Treatments were control (containing MiyaGold) and groups fed the same control diet supplemented with lasalocid sodium (75 mg kg^{-1}), salinomycin sodium (50 mg kg^{-1}), calcium halofuginone polystyrenesulfonate (40 mg kg^{-1}) and decoquinate (40 mg kg^{-1}). However, the tested concentration of lasalocid sodium (75 mg kg^{-1}) and salinomycin (50 mg kg^{-1}) were lower than the maximum authorised levels (125 mg $kg^{-1}$and 70 mg kg^{-1}, respectively). Moreover, halofuginone was supplied in a form not authorised in Europe and at a dose which is difficult to relate to the authorised level.

Animals were sacrificed on day 10, caecal contents were harvested and both vegetative and spore counts of clostridia in caecal contents were determined. Enumeration of *C. butyricum* was performed using a selective media and an immunological assay based on monoclonal antibodies (Sato and Tanaka, 1997).

The caecal counts of vegetative cells and spores were almost equivalent between control and coccidiostats-treated groups, varying by no more than ±1.0 log order compared to the control (Table 3).

Table 3. ***Clostridium* caecal counts in chickens for fattening receiving coccidiostats**

Miya-Gold® S (CFU kg^{-1}feed)	Coccidiostats	Total counts (log_{10} CFU/g)	Spores (% of total count)
5 x 10^8	none	3.8	38.8
5 x 10^8	lasalocid 75 mg kg^{-1}	3.6	49.0
5 x 10^8	salinomycin 50 mg kg^{-1}	3.8	56.2
5 x 10^8	halofuginone 40 mg kg^{-1}	4.0	55.6
5 x 10^8	decoquinate 40 mg kg^{-1}	4.0	20.6

A second *in vivo* study aiming to demonstrate the compatibility of Miya-Gold®S with monensin, diclazuril, maduramycin, robenidine, narasin, narasin/nicarbazin, semduramycin and formic acid was provided.[22]

[20] Technical dossier/Annex 3-2
[21] Technical dossier/Annex 2-3-3
[22] Supplementary dossier, February 2009/Final M105 Report

Chickens (12 birds per treatment) were housed in cages (six birds per cage) and fed control diet or the control diet supplemented with coccidiostats or formic acid as shown in Table 3. Miya-Gold®S was supplemented at the recommended dose of 5 x 10^8 CFU kg^{-1} and the coccidiostats at the maximum EU authorised levels. Formic acid was applied at a typical dose of 0.5 %. The concentrations of coccidiostats, formic acid and CBM 588 were confirmed in feeds by analysis. The experiment lasted 14 days.

Caecal contents from birds were harvested and subjected to differential counts of vegetative cells and spores. Enumeration of *C. butyricum* was performed using a selective media and an immunological assay based on monoclonal antibodies (Sato and Tanaka, 1997). *C. butyricum* CBM 588 was not detected in the caecal content of animals from the negative control without Miya-Gold®S while it was detected in the gut contents of all the other treatments, both as viable vegetative and spores forms (Table 4). The counts of *C. butyricum* CBM 588 were equivalent between treatments, differing by no more than ±1.0 log order, compared to the Miya-Gold®S control (Table 4).

Table 4. **Caecal *Clostridium* counts in chickens for fattening receiving coccidiostats and formic acid**

Miya-Gold® S (CFU kg^{-1}feed)	Coccidiostats/ organic acid	Vegetative cells (log_{10} CFU/g)	Spores counts (log_{10} CFU/g)
0	none	Not detected	Not detected
5 x 10^8	none	3.69	3.39
5 x 10^8	monensin (125 mg kg^{-1})	3.32	3.17
5 x 10^8	diclazuril (1 mg kg^{-1})	3.92	3.14
5 x 10^8	maduramycin 5 mg kg^{-1}	3.27	3.10
5 x 10^8	robenidine 36 mg kg^{-1}	3.70	3.09
5 x 10^8	sarasin 70 mg kg^{-1}	3.23	3.08
5 x 10^8	narasin 50 mg kg^{-1}/ nicarbazin 50 mg kg^{-1}	3.15	3.10
5 x 10^8	semduramicin 25 mg kg^{-1}	3.39	3.25
5 x 10^8	formic acid 0.5 %	3.37	3.20

3.2. Conclusions on efficacy

The applicant provided four trials showing evidence of a significant improvement in feed to gain ratio in chickens for fattening receiving Miya-Gold®S at the proposed dose of 5 x 108 CFU kg-1 feedingstuff. Therefore, the FEEDAP Panel considers that evidence of efficacy of Miya-Gold®S has been demonstrated at the recommended dosage.

Compatibility has been demonstrated in in vivo studies with the coccidiostats monensin, diclazuril, maduramycin, robenidine, narasin, narasin/nicarbazin, semduramycin and decoquinate at the maximum authorised concentrations for chickens for fattening. Compatibility has also been demonstrated with lasalocid sodium and salinomycin sodium at concentrations lower than the maximum authorised levels. The data provided by the applicant support the compatibility with formic acid at 0.5 % in feed.

4. Safety

4.1. Safety for chickens for fattening

A tolerance test associated with efficacy study 1 was provided by the applicant.[23] Animals were divided in four groups: control and three treatment groups, providing Miya-Gold®S kg^{-1} feed at doses of 5 x 10^8, 5 x 10^9 and 5 x 10^{10} (100X the recommended dose) CFU kg^{-1} complete feed,. The study was made using diclazuril and monensin in grower (1-21 days of age) and finisher (22-42 days of age) periods, respectively.

The zootechnical parameters of one-day-old to 42-day-old animals were recorded. Blood samples were obtained from ten males and ten females per treatment at the end of the trial (day 43), and haematological (haematocrit, haemoglobin, mean corpuscular haemoglobin concentration, leucocytes and differential leucocyte counts) and biochemical parameters (serum aspartate amino transferase, alanine aminotransferase, gammaglutamine transpeptidase, uric acid, albumin and total proteins) were analysed. The data were analysed using an ANOVA test.

Mortality was less than 2 % and not treatment-related. Performance was not adversely affected by the overdose of Miya-Gold®S (see Table 2). None of the blood parameters were significantly affected by the inclusion of CBM 588 in the feed.

Faecal samples were taken at 21 and 42 days of age. The counts of CBM 588 increased in faeces as the dosage of Miya-Gold®S increased in feed. No significant differences were detected between treatments for *C. perfringens* and *E. coli* counts on faecal samples taken on day 42.

4.1.1. Conclusions on safety for the target species

As no adverse response was seen when Miya-Gold®S was included in diets at 100 times the recommended dose, the FEEDAP Panel concludes that the product is safe for chickens for fattening at the recommended dose.

4.2. Safety for the consumer

The active agent of Miya-Gold®S is a strain of *C. butyricum*, which has been demonstrated to lack genes coding for known clostridial toxins. Moreover, this strain is susceptible to all the antibiotics defined by the Technical guidance on the update of the criteria used in the assessment of bacterial resistance to antibiotics of human or veterinary importance (EFSA, 2008).

4.2.1. Acute oral toxicity

C. butyricum Miyairi powder CBM 588 was administered by gavage as a suspension in aqueous carboxymethylcellulose to groups of ten rats of each sex at a dose 5000 mg kg^{-1}.[24] After treatment, the animals were observed for 14 days. The animals were examined grossly at necropsy. There were no changes attributable to the administration of the product.

4.2.2. Genotoxicity

The applicant has provided a bacterial reverse mutation assay and a chromosomal aberrations assay in mammalian cells made with a lysate of the product strain (*C. butyricum* CBM 588). Both showed negative results at the maximum dose recommended for such studies. The

[23] Tecnical dossier/Annex 4.1.1
[24] Technical dossier/Annex 4.2.2.1

FEEDAP Panel notes that the use of cell lysates is of limited value since in principle it does not take account of possible toxic compounds produced during the fermentation and carried over into the final product. However, in this specific case, the FEEDAP Panel notes that the spore suspension was washed and that production of known toxins was excluded.

4.2.3. Sub-acute oral toxicity

A five-week sub-acute toxicity test was performed with Miya-Gold®S. It was given in capsules for five weeks to groups of three beagle dogs providing doses of 80, 400 and 2000 mg kg^{-1} day^{-1}.[25] Two additional animals of each sex were treated at control and high-dose level and were allowed to continue untreated for a further five weeks after the end of the treatment period. Clinical observations, ophthalmoscopy, body weight, feed and water consumption were recorded during the study. Urine was collected for analysis and blood samples were taken for haematological and clinical chemistry measurements on three occasions (start, five and ten weeks). At necropsy organ weights (brain, pituitary, thyroid, thymus, heart, lungs, liver, kidney, adrenal, spleen, testes or ovaries) were measured and all animals examined for gross abnormalities. A wide range of tissue samples were collected at necropsy and were examined for evidence of histological changes.

No deaths were observed. Although some differences were seen between the groups there were no differences at any dose which indicated an adverse effect of treatment with Miya-Gold®S.

4.2.4. Chronic oral toxicity

Miya-Gold®S was administered in the diet to groups of 20 rats of each sex at a level of 500, 5000 and 50000 mg kg^{-1} diet for 52 weeks.[26] The resulting doses were calculated to be 0, 24, 241 and 2411 mg kg^{-1} day^{-1} for males and 0, 29, 288 and 2953 mg kg^{-1} day^{-1} for females. All animals were observed twice a day; body weight and feed intake were measured weekly. Ophthalmoscopy was conducted at start and at 26 and 52 weeks of treatment. Urinalysis was performed at both 26 and 52 weeks while blood samples were taken for haematology and clinical chemistry only at 52 weeks after fasting. At the end of the treatment, a full necropsy was conducted with measurement of organ weights and collection of tissues for microscopic examination.

There was no mortality during the study and there was no difference in the clinical observations between treatments. Most observations showed no differences which could be attributed to treatment. However, at necropsy, the absolute and relative weights of livers of both sexes were lower than those of controls. The histological examination revealed no differences between treated and control groups. Since the difference in liver weight seen at the highest dose appears to be linked to treatment, the NOAEL concluded from this study is 241 or 288 mg kg^{-1} day^{-1} for males and females, respectively.

4.2.5. Conclusions on consumer safety

The specific assays provided exclude the potential for production or presence of known clostridium toxins in the product strain. In addition, results of the toxicity studies provided do not indicate any reason for concerns arising from the use of Miya-Gold®S as an additive in animal feed.

[25] Technical dossier/Annex 4.2.2.2
[26] Technical dossier/Annex 4.2.2.3

4.3. Safety for the user

An acute dermal irritation study in three rabbits in accordance with OECD Guideline 404 was conducted with 0.5 g of Miya-Gold®S and total absence of any response indicates that this preparation is non-irritant.[27]

In an acute eye irritation study in rabbit, conducted according to OECD Guideline 405, with Miya-Gold®S, there was a slight oedema and erythema immediately after treatment which had disappeared by 24 hours. Thus the product is classified as non-irritant.[28]

No acute inhalation or skin sensitisation tests have been provided. As the particle size data indicate a significant proportion of the product is potentially respirable, sensitisation via respiratory route cannot be excluded.

4.3.1. Conclusions on user safety

The product is non-irritant to skin and eyes. The significant proportion of the product with a particle size ~10 μm indicates significant potential for inhalation exposure which, combined with the proteinaceous nature of the product, is considered to represent a risk of respiratory sensitisation.

4.4. Safety for the environment

C. butyricum is a natural component of soil microbiota and its use as Miya-Gold®S in animal feeding would not be expected to pose a risk for the environment.

5. Post-market monitoring

No risks associated with the use of the product are foreseen. It is considered that there is no need for specific requirements for a post-market monitoring plan other than those established in the Feed Hygiene Regulation[29] and Good Manufacturing Practice.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

A significant improvement in feed to gain ratio was observed in four studies when chickens for fattening were fed Miya-Gold®S at 5 x 10^8 CFU kg^{-1} feedingstuff. Therefore, the FEEDAP Panel considers that evidence of the efficacy of Miya-Gold®S has been demonstrated at the recommended dose.

The compatibility of Miya-Gold®S with the coccidiostats monensin, diclazuril, maduramycin, robenidine, narasin, narasin/nicarbazin, semduramycin and decoquinate at the maximum authorised concentrations and with formic acid (0.5 %) has been demonstrated. Compatibility with lasalocid sodium and salinomycin sodium has been observed only at concentrations lower than the maximum authorised levels.

As no adverse response was seen when Miya-Gold®S was included in diets at 100 times the recommended dose, the FEEDAP Panel concludes that the product is safe for chickens for fattening at the recommended dose.

[27] Supplementary dossier December 2008/Annex SD 4.3.1.1
[28] Supplementary dossier December 2008/Annex SD 4.3.1.2
[29] OJ L 35, 8.2.2005, p.1

The FEEDAP Panel considers that the use of Miya-Gold®S as a feed additive would not pose a risk for the consumer. The strains *C. butyricum* CBM 588 lacks toxigenic potential and does not harbor acquired antibiotic resistances.

The concerns for the safety of the users are limited to possible sensitisation.

The use as feed additive of *C. butyricum*, a naturally occurring soil micro-organism, is not considered to pose a risk to the environment.

RECOMMENDATIONS

The FEEDAP Panel recommends that:

- the product strain should also be deposited in a recognised European culture collection.
- if losses on pelleting of 50 % are typical for this product, then this should be indicated on the label.
- Miya-Gold®S should be considered as a respiratory sensitiser and treated accordingly.

DOCUMENTATION PROVIDED TO EFSA

1. Miya-Gold®S. April 2008. Submitted by PenTec Consulting.
2. Supplementary dossier, November 2008. Submitted by PenTec Consulting.
3. Supplementary dossier, February 2009. Submitted by PenTec Consulting.
4. Evaluation report of the Community Reference Laboratory for Feed Additives on the methods(s) of analysis for Miya-Gold®S.
5. Comments from Member States received through the ScienceNet.

REFERENCES

EFSA (European Food safety Authority), 2008. Technical guidance. Update of the criteria used in the assessment of bacterial resistance to antibiotics of human or veterinary importance. Prepared by the Panel on Additives and Products or Substances used in Animal Feed. <http://www.efsa.europa.eu/cs/BlobServer/Scientific_Opinion/feedap_op_ej732_tg_antimicrobial_resistance_en.pdf?ssbinary=true>

Sato R. and Tanaka M, 1997. Intestinal Distribution and Intraluminal Localization of Orally Administered *Clostridium butyricum* in Rats. Microbiol. Immunol. 41(9), 665-671.

APPENDIX

Executive Summary of the Evaluation Report of the Community Reference Laboratory for Feed Additives on the Method(s) of Analysis for Miya-Gold®S

In the current application authorisation is sought for the microbial product Miya-Gold®S as a feed additive under the category 'zootechnical additives', functional group 'gut flora stabilisers' according to Annex I of Regulation (EC) No 1831/2003. Specifically, the use of Miya-Gold®S for chickens for fattening is requested. Miya-Gold®S contains 5.0 x 10^8 viable cells (c.f.u., colony-forming units) of *Clostridium butyricum* MIYAIRI 588 (as active agent) per gram. The feed additive is intended to be mixed into complete feedingstuffs at final concentrations of 5 x 10^8 c.f.u./kg.

For the quantification of the active agent, *Clostridium butyricum* MIYAIRI 588, in the *feed additive* and *premixtures* an enumeration method using iron sulfite agar as described in ISO Standard 15213 (2003) is proposed by the applicant. For *feedingstuffs* the applicant proposes a selective CMB588 agar (Microbiol. Immunol. 1997, 41(9), 665-671). A three-laboratory validation study demonstrated satisfactory performance of the iron sulfit agar and selective CMB588 agar using samples of the additive, premixtures and feedingstuffs. The method performance characteristics were standard deviations for repeatability (s_r) and reproducibility (s_R) of below 0.10 $\log_{10}$ and of between 0.09 – 0.31 $\log_{10}$ calculated from the base 10 logarithms of the measured c.f.u./g, respectively. Presumptive colonies of *Clostridium butyricum* MIYAIRI 588 shall be confirmed microscopically, for absence of growth under aerobic conditions and for formation of butyric acid on plate count agar.

For official controls of the active agent *Clostridium butyricum* MIYAIRI 588 in the *feed additive* and *premixtures* iron sulfite agar according to ISO 15213 and in *feedingstuffs* the selective CMB588 agar (Microbiol. Immunol. 1997, 41(9), 665-671) are recommended followed by confirmation of presumptive *Clostridium butyricum* MIYAIRI 588 colonies on plate count agar. The CRL-FA recommends using for analysis of premixtures 20 g samples for feedingstuffs 50 g.

The limits of quantification (LOQ) of the spread plate methods are around 10^4 colony forming units (c.f.u) per gram (g) feed additive or premixture and around 10^7 c.f.u./kg feedingstuff which is well below the anticipated target concentrations

The applicant applied a wide range of biochemical and molecular techniques for strain identification of *Clostridium butyricum* MIYAIRI 588 including pulsed field gel electrophoresis (PFGE) [Jpn. Pharmacol. Ther. 2000, 28(12), 999-1004]. PFGE is widely applied for microbial strain identification and is considered suitable for official controls in the frame of the authorisation.

On the basis of the supplied documentation, no supplementary experimental work (testing or method validation) is required.