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Safety and efficacy of BIOSTRONG® 510 (essential oil of thyme and star anise) for chickens and minor avian species for fattening and rearing to point of lay

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)

Abstract

Following a request from the European Commission, the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety and efficacy of the product BIOSTRONG® 510 (essential oil from thyme and star anise, crushed dried spices and crushed dried herbs), when used as a feed additive for chickens for fattening and rearing to point of lay and minor avian species for fattening and rearing to point of lay. BIOSTRONG® 510 is a preparation of partially microencapsulated essential oils from thyme and star anise, dried herbs and dried spices. The FEEDAP Panel concludes that BIOSTRONG® 510 is safe for chickens for fattening at the proposed conditions of use and that this conclusion can be extended to chickens reared for laying and extrapolated to all minor poultry species for fattening and reared to point of lay. The FEEDAP Panel concludes that the use of BIOSTRONG® 510 as an additive in the feed for chickens for fattening does not present risk for the consumer of meat and meat products. Irritancy studies have not been provided, however, because of the content of saponins, the FEEDAP Panel assumes that the additive is highly irritant to mucous membranes, and considers that measures to minimise exposure by all routes are necessary for the handling of this product. The use of BIOSTRONG® 510 at the recommended levels is not considered to be a risk for the environment. The FEEDAP Panel considers that BIOSTRONG® 510 has the potential to be efficacious in improving performance of chickens for fattening. This conclusion can be extended to chickens reared for laying and extrapolated to all minor poultry species for fattening and reared to point of lay at the same dose.

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Keywords: zootechnical additive, BIOSTRONG® 510, chickens for fattening and reared for laying, safety, efficacy

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Correspondence: feedap@efsa.europa.eu

Panel members: Gabriele Aquilina, Vasileios Bampidis, Maria de Lourdes Bastos, Georges Bories, Andrew Chesson, Pier Sandro Cocconcelli, Gerhard Flachowsky, Jürgen Gropp, Boris Kolar, Maryline Kouba, Secundino López Puente, Marta López-Alonso, Alberto Mantovani, Baltasar Mayo, Fernando Ramos, Guido Rychen, Maria Saarela, Roberto Edoardo Villa, Robert John Wallace and Pieter Wester

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Note: This scientific opinion has been amended following the adoption of the decision of the Commission on confidentiality claims submitted by the applicant, in accordance with Article 8(6) and Article 18 of Regulation (EC) No 1831/2003. The abstract has been modified, as have other sections as indicated in the text.

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1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003[1] 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 Delacon Biotechnik GmbH[2] for authorisation of the product BIOSTRONG® 510 (essential oil from thyme and star anise, crushed dried spices and crushed dried herbs), when used as a feed additive for chickens for fattening and rearing to point of lay and minor avian species for fattening and rearing to point of lay (category: zootechnical additives; functional group: digestibility enhancers, other zootechnical additives).

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. 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 5 January 2012.

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 safety for the target animals, consumer, user and the environment and on the efficacy of the product BIOSTRONG® 510, when used under the proposed conditions of use (see Section 3.1.6).

1.2. Additional information

BIOSTRONG® 510 is an additive consisting of essential oil from thyme and a synthetic mixture mimicking star anise oil, crushed dried spices and crushed dried herbs. This product has not been previously authorised in the European Union (EU).

BIOSTRONG® 510 was initially formulated with star anise essential oil. Subsequently, the applicant replaced the star anise oil with a synthetic mixture to avoid the presence of the known carcinogen estragole in star anise oil (EC, 2001).

Thymol, the major component in thyme oil, and *trans*-anethole, the major constituent of star anise synthetic mixture, have been assessed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA; WHO, 2000, 2001, 2005a) and were considered safe for use in food. An acceptable daily intake (ADI) value was set for *trans*-anethole. The EFSA Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) considered thymol for use as food flavouring with similar conclusions (EFSA, 2008a). EFSA did not express an opinion on *trans*-anethole because it was not included in the evaluation programme (Regulation (EC) No 1565/2000)[3]. The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) assessed thymol and *trans*-anethole in two opinions and concluded that their use in feed of thymol and *trans*-anethole is safe up to 5 and 25 mg/kg feed, respectively (EFSA FEEDAP Panel, 2011a, 2012a).

Thyme oil and star anise oil are included in the EU Register of Feed Additives following the provisions of Article 10(1) of Regulation (EC) No 1831/2003 as sensory additives, functional group: flavouring compounds.

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier[3] in support of the authorisation request for the use of BIOSTRONG® 510 as a feed additive. The

[1] Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.
[2] Delacon Biotechnik GmbH, Weissenwolffstr. 14, 4221, Steyregg, Austria.
[3] FEED dossier reference: FAD-2011-0036.

technical dossier was prepared following the provisions of Article 7 of Regulation (EC) No 1831/2003 and the applicable EFSA guidance documents.

The FEEDAP Panel has sought to use the data provided by the applicant together with data from other sources, such as previous risk assessments by EFSA or other expert bodies, peer-reviewed scientific papers and experts' knowledge, to deliver the present output.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the active substance thymol in animal feed. The Executive Summary of the EURL report can be found in Annex A.[4]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of BIOSTRONG® 510 is in line with the principles laid down in Regulation (EC) No 429/2008[5] and the relevant guidance documents: Guidance for the preparation of dossiers for zootechnical additives (EFSA FEEDAP Panel, 2012b), Guidance for the preparation of dossiers for sensory additives (EFSA FEEDAP Panel, 2012c), Technical guidance: Tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011b), Technical Guidance for assessing the safety of feed additives for the environment (EFSA, 2008b), Guidance for establishing the safety of additives for the consumer (EFSA FEEDAP Panel, 2012d), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012e).

3. Assessment

3.1. Characterisation

3.1.1. Characterisation of the additive[6]

BIOSTRONG® 510 is a beige-brownish powder, consisting of the essential oil of thyme and the mixture of pure compounds mimicking star anise oil (referred to as star anise oil PC), partially microencapsulated, quillaja bark powder, crushed dried herbs and spices, and other excipients. The product specifications are based on the main components. The applicant provides a specification for thymol (2–4 mg/g) and *trans*-anethole (40–50 mg/g).[7] Analysis of five batches of the additive showed compliance with these specifications. Analyses of thymol (mean value 2.92 mg/g, range: 2.9–3.0 mg/g) and *trans*-anethole (mean value 45.4 mg/g, range: 44.0–47.5 mg/g)[8] showed the minimum specification was exceeded in all cases. Quillaja bark (*Quillaja saponaria*) contains about 10% saponins, which consist primarily of glycosides of quillaic acid (quillaja sapogenin, hydroxygypsogenin). The saponin content in the final product is specified as $\leq$ 23 mg/g (average in eight batches 20 mg/g, range: 19–22 mg/g; certificates of analysis were not provided).

In order to exclude the presence of estragole contained in star anise oil, a mixture of pure compounds was created containing the major components present in the natural oil in comparable quantities. This was confirmed by chromatographic analysis. Analysis of five batches of the star anise oil PC[9] and the reformulated additive[10] did not detect estragole (limit of quantification (LOQ) $<$ 1 and $<$ 5 μg/g, respectively).

The applicant routinely monitors the final product for impurities, including heavy metals and arsenic, pesticides and microbial contamination. Data from three batches showed that the levels of cadmium, lead, arsenic and mercury were between 0.15 and 0.18, 0.70 and 1.00, 1.8 and 2.7 and $<$ 0.005 and 0.005 mg/kg, respectively, and considered not to be of concern.

Because the additive contains plant materials, a full pesticide residue analysis was made of three batches. The levels were below the limit of detection (LOD, 0.005–0.01 mg/kg), except for pirimiphos-

[4] The full report is available on the EURL website: http://irmm.jrc.ec.europa.eu/SiteCollectionDocuments/FinRep-FAD-2011-0036.pdf

[5] Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1.

[6] This section has been amended following the provisions of Article 8(6) and Article 18 of Regulation (EC) No 1831/2003.

[7] Technical Dossier/Supplementary information July 2015.

[8] Technical Dossier/Supplementary information July 2015/Annex II.1.3.5.

[9] Technical dossier/Supplementary information July 2015/Annex II.1.3.3.

[10] Technical dossier/Supplementary information July 2015/Annex II.1.3.4.

methyl (0.017–0.020 mg/kg) in all three batches and cypermethrin (0.028 mg/kg) in one batch. Total polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans (PCDD/Fs) were between 0.11 and 0.12 ng WHO-PCDD/F-TEQ (toxic equivalents)/kg. The mycotoxins analysed were zearalenone < 10 μg/kg, deoxynivalenol 70 μg/kg, ochratoxin A 0.8–1.2 μg/kg and the sum of aflatoxins < 2 μg/kg. These values do not raise safety concerns.

Microbiological quality was addressed with the same three batches of the additive. *Salmonella* (colony forming unit (CFU)/25 g) was not detected, *Bacillus cereus* was between 5 and 20 × 10^2 CFU/g, while *Staphylococcus aureus*, sulfite-reducing clostridia, Enterobacteriaceae and *Escherichia coli* were < 10 CFU/g.[11]

Particle size measured by laser diffraction (one batch) ranged from 4.5 to 515 μm. Approximately, 50% of particles exhibited a diameter of 100 μm or less and around 12% of particles had a particle size diameter smaller than 10 μm.[12] Stauber–Heubach data of three batches indicate a high dusting potential of up to 3.4 g/m^3 (range: 3.0–3.4 g/m^3).[13] The bulk density is 500 kg/m^3 [8] and the density is 1,000 kg/m^3.[12]

3.1.2. Characterisation of the active substances[6]

The active substances in BIOSTRONG® 510 predominantly derive from the thyme oil, star anise oil PC and quillaja bark. The crushed herbs and spices will also contribute to the activity but to a lesser extent.

Essential oil of thyme from *Thymus vulgaris* L. is extracted by steam distillation.[14] According to the European Pharmacopoeia (PhEur), the components identified in thyme oil are thymol (36–55%), *p*-cymene (15–28%), γ-terpinene (5–10%), linalool (4–6.5%), carvacrol (1–4%), β-myrcene (1–3%) and terpinen-4-ol (0.2–2.5%) (PhEur, 2005). The applicant provided the composition of Star Anise oil PC.

The applicant did not provide information on the manufacturing process of quillaja bark powder. Quillaja bark powder contains about 10% saponins, which consist primarily of glycosides of quillaic acid (quillaja sapogenin, hydroxygypsogenin). A content of tannins to a maximum of 8% dry matter has been reported for quillaja bark extracts (WHO, 2005b).

3.1.3. Manufacturing process of the additive[6]

The applicant provided a detailed description and a flow chart of the manufacturing process of the additive BIOSTRONG® 510.

3.1.4. Stability and homogeneity

The stability of the additive itself and feed containing the additive was assessed by monitoring the content of thymol[15] and *trans*-anethole.[16]

The shelf life of the additive was studied in three batches at 25°C/60% relative humidity (RH) for 18 months and at 40°C/75% RH for 6 months. Losses of around 14–19% for thymol[16] and 1–17% for *trans*-anethole[17] were observed under 25°C and around 27–30% losses of thymol[16] in the 40°C/75% RH conditions.

The stability of BIOSTRONG® 510 was tested in three batches of a vitamin/mineral premixture containing choline chloride. After 6 months, losses were of about 32–38% for thymol[16] and 18–24% for *trans*-anethole[17] when stored at 25°C, and 38–43% for thymol[16] when stored at 40°C.

The stability of BIOSTRONG® 510 in mash feed was tested in three batches. After 4 months, losses were of about 15–35% for thymol[16] and 22–36% for *trans*-anethole[17] when stored at 25°C, and 40–49% for thymol[16] when stored at 40°C. In pelleted feed after 4 months, losses were between 24% and 34% for thymol[16] and 1% and 18% for *trans*-anethole[17] at 25°C, and between 31% and 50% for thymol[19] at 40°C. No data were submitted on the effect of feed processing.[16,17]

[11] Technical Dossier/Section II/Annex II.1.4.
[12] Technical Dossier/Section II/Annex II.1.5.1.
[13] Technical Dossier/Section II/Annex II.1.5.2.
[14] Technical Dossier/Section II/Identity.
[15] Technical Dossier/Section II/Annex II.4.1.2.
[16] Technical Dossier/Supplementary information May 2012/Annex II.4.1.3.
[17] Technical Dossier/Section II/Annex II.4.2.

These data indicate that the concentration of the compounds thymol and *trans*-anethole decreases with time in the additive, premixtures, mash and pelleted feed.

The homogeneous distribution of the additive in a premixture and a complete feedingstuff was assessed by measuring the thymol content in 10 subsamples each of a premixture and a mash and pelleted feed.[17] The coefficients of variation were 4.4% in premixtures, 4.8% in mash feed and 2.8% in pelleted feed.

3.1.5. Physicochemical incompatibilities or interactions

Based on current knowledge, no incompatibilities resulting from the use of the product in compound feed are expected with other feed materials, carriers, other approved additives or medicinal products.

3.1.6. Conditions of use

BIOSTRONG® 510 is intended for use in feed for chickens for fattening, chickens reared for laying and minor species for fattening and reared to point of lay at a dose of 150 mg/kg complete feedingstuffs.

3.2. Safety

All of the studies related to safety were made with the original formulation containing natural star anise oil. Given the degree of similarity of the mixture (star anise oil PC) and the natural oil, the FEEDAP Panel considers that the safety studies made with the original formulation can be used to assess the safety of the new formulation.

3.2.1. Safety for the target species

Two tolerance studies in chickens for fattening with BIOSTRONG® 510 were provided.

In the first study, 576 1-day-old male Cobb 500 chickens were distributed into 24 pens and allocated to one of four dietary treatments, resulting in six replicates of 24 chickens per treatment.[18] Feed and water were available *ad libitum* over an experimental period of 42 days. The starter diet (pelleted feed) was offered from day 1 until day 21 and the finisher diet (pelleted feed) from day 22 until day 42 of the experiment. BIOSTRONG® 510 was incorporated into a basal maize–wheat–soybean meal at 0, 150 (× 1 recommended dose), 750 (× 5) and 1,500 (× 10) mg/kg of feed. Confirmation of the dose was made by analysis of the thymol content of the feed (0.01, 0.45, 1.90 and 4.16 mg/kg thymol, respectively, for the starter feed). Mortality and zootechnical performance (average daily feed intake, body weight, average daily gain and feed to gain ratio) were recorded. In addition, routine blood haematology and biochemistry were carried out, at 35 days of age, on 12 birds per treatment (two per pen).[19] At 42 days of age, 12 birds per treatment (the same that had been selected for blood analysis) were killed for gross pathology examination. An analysis of variance (ANOVA) was performed with the data considering the pen as the experimental unit. Differences were considered significant at a level of at least $p < 0.05$.

The birds remained healthy throughout the study. The overall mortality rate was low (1.04%). Inclusion of BIOSTRONG® 510 at any dose tested did not significantly affect average daily feed intake (101–106 g/day), final body weight (2.9–3.0 kg) and average daily gain (69.2–71.3 g/day). A dose-dependent improvement ($p < 0.001$) of feed to gain ratio (1.53, 1.48, 1.44 and 1.42 for the treatments of 0, 150, 750 and 1,500 mg BIOSTRONG® 510/kg feed, respectively) was recorded for the overall experimental period. Blood haematology and biochemistry (35th day of age), and gross pathology at post-mortem (42nd day of age) examination, did not reveal any sign of toxicity.

In a second study, 432 1-day-old male and female Ross 308 chickens were distributed into 36 pens and allocated to one of three dietary treatments, resulting in 12 replicates of 12 chickens per treatment (equal distribution of male and female chickens).[20] Feed and water were available *ad libitum* over an experimental period of 35 days. The starter diet (mash feed) was offered from day 1 until day 14, the grower diet (mash feed) was offered from day 15 until day 28 and the finisher diet (mash

[18] Technical Dossier/Section III/Annex III.1.2.Conf.

[19] White and red blood cells, lymphocytes, haemoglobin, haematocrit, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), electrolytes (sodium, potassium, chloride, calcium and phosphorus), alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total cholesterol, triglycerides, uric acid, bilirubin, glucose, total protein and albumin.

[20] Technical Dossier/Section III/Annex III.1.1.Conf.

feed) from day 29 until day 35 of the experiment. BIOSTRONG® 510 was incorporated into a basal maize–wheat–soybean meal at 0, 150 (× 1 recommended dose, 0.29, 0.28 and 0.27 mg/kg thymol, analysed for starter, grower and finisher diets, respectively) and 1,500 (× 10, 3.00, 2.54 and 2.87 mg/kg thymol, analysed for starter, grower and finisher diets, respectively) mg/kg. The analysed thymol content was lower by 50% compared to the theoretical thymol content. Mortality and zootechnical performance (average daily feed intake, body weight, average daily gain and feed to gain ratio) were recorded. At 35 days of age, 72 chickens (24 from each treatment group, gender not specified) were killed and the carcass characteristics evaluated. In addition, meat and liver samples of six chickens per treatment (3 males and 3 females) were taken in order to analyse possible residues of thymol (meat), *trans*-anethole (meat) and α-pinene (meat and liver).[21] An ANOVA was made with the performance data considering the pen as the experimental unit, except for the residue analysis where individual chicken was the experimental unit. Differences were considered significant at a level of at least $p < 0.05$.

The overall mortality rate was low (2.8%). Inclusion of BIOSTRONG® 510 at any dose tested did not significantly affect average daily feed intake (94.0–96.7 g/day), final body weight (2.1–2.2 kg), average daily gain (60.6–60.9 g/day) and feed to gain ratio (1.56–1.59). Carcass composition data at slaughter did not reveal negative effects of BIOSTRONG® 510 on carcass quality. Thymol, *trans*-anethole and α-pinene were not detected in meat samples (respective LODs: 0.01, 0.1 and 0.1 μg/g thymol, *trans*-anethole and α-pinene) and α-pinene was not detectable in liver samples (LOD: 0.1 μg/g liver).[22]

3.2.1.1. Conclusions on safety for the target species

Chickens for fattening tolerated a 10-fold overdose of BIOSTRONG® 510, as shown in the tolerance study, without any adverse effects on health, performance or blood parameters. This is supported by the observation of another trial in which no adverse effects on performance parameters were observed.

Therefore, the FEEDAP Panel concludes that BIOSTRONG® 510 is safe for chickens for fattening at the proposed dose of 150 mg/kg complete feedingstuffs. The FEEDAP Panel considers that this conclusion can be extended to chickens reared for laying and extrapolated to all minor poultry species for fattening or reared to point of lay at the same dose.

3.2.2. Safety for the consumer

The assessment of the consumer safety is based on reported composition of the ingredients of the additive.

- Thyme oil and star anise oil[6]

Thymol and *trans*-anethole, the two major constituents of thyme oil and star anise oil PC, are currently authorised for use in food without limitations.

Thymol [FLAVIS-no: 04.006] has been evaluated by JECFA (WHO, 2004a) and EFSA (EFSA, 2008b) as food flavour. The FEEDAP Panel considered that the use of thymol as flavour in all animal species up to the highest use level proposed in feed (5 mg/kg) is safe for the consumer (EFSA FEEDAP Panel, 2012a).

trans-Anethole [04.010] has been evaluated by JECFA (WHO, 2000) that established an ADI of 0–2 mg/kg body weight (bw) per day. This ADI was based on a 90-day study in rats showing alteration in serum parameters considered to be indicative of hepatotoxicity and a safety factor of 200, taking into account the deficiencies of the studies considered. In its opinion on *trans*-anethole as a feed flavouring for all animal species, the FEEDAP Panel considered that its use up to the highest use level proposed in feed (25 mg/kg) is safe for the consumer (EFSA FEEDAP Panel, 2011a).

The maximum declared values of thymol (4 mg/g) and *trans*-anethole (50 mg/g) content in the additive were considered in the assessment. The use of BIOSTRONG® 510 at a level of 150 mg/kg feed will result in a maximum content of 0.6 mg thymol/kg and 7.5 mg *trans*-anethole/kg in complete feed. These levels are lower than the feed concentrations considered safe for consumers (EFSA FEEDAP Panel, 2011a, 2012a).

[21] Technical Dossier/Section III/Annex III.1.1.Conf. and Supplementary information May 2012/Annex III.1.3. and Supplementary information May 2013/Annex III.2.1.2.
[22] Technical Dossier/Section III/Annex III.1.1.Conf. and Supplementary information May 2012/Annex.III.1.3.

Analyses of meat and liver samples from six chickens fed BIOSTRONG® 510 for 5 weeks at the maximum recommended dose (150 mg/kg feed) and 10-fold the maximum dosage (1,500 mg/kg feed) did not detect any thymol or *trans*-anethole residues (see second tolerance study).[22]

The concentrations of the other components in feed supplemented with 150 mg BIOSTRONG® 510/kg were calculated considering the maximum value of the concentration range reported in Section 3.1.2. The resulting concentrations were compared with the ones considered as safe in previous assessments of the individual substances used as feed flavourings in feed for poultry (EFSA FEEDAP Panel, 2012a,f,g, 2015, 2016). Linalool is present in both thymol and star anise oil; therefore, the concentrations were summed up.

cis-Anethole have not been previously assessed by EFSA. The FEEDAP Panel noted that a no adverse effect level of 172 mg/kg per day was derived from a chronic study in rat (Truhaut et al., 1989).[23] However, the Panel did not have access to this study and concluded on a safe level using the threshold of toxicological concern (TTC) approach.

For all compounds the levels in feed supplemented with BIOSTRONG® 510 at 150 mg/kg are considerably lower than the feed concentrations which were considered safe for consumers.

- Quillaja bark powder

Quillaja bark (*Quillaja saponaria*) contains about 10% saponins, which consist primarily of glycosides of quillaic acid (quillaja sapogenin, hydroxygypsogenin). Saponins are strongly irritating to mucous membranes and can cause haemolysis and tissue damage after absorption (Westendorf, 1999). Small quantities of quillaja bark extracts (up to 200 mg/kg) are added as foaming agents to carbonated beverages and other foodstuffs (WHO, 2005b) and for pharmaceutical purposes as an absorption enhancer of drugs (Recchia et al., 1995). No adverse effects were observed in a study on human volunteers orally administered a drink containing 0.5 mg quillaja saponins/kg bw per day for 7 days (Naknukool et al., 2011). An ADI of 1 mg kg/bw was established by the Australian Food Standard Agency for quillaja extract (2013).

Considering the quantity fed to chickens (0.36 mg saponins/day) and the poor absorption of saponins in the gastrointestinal tract (Cheeke, 1989), and the fact that it will not be deposited in tissue and that no residues are expected, neither there will be an exposure of consumers, toxic effects of quillaja saponins are not expected to occur after consumption of food prepared from animals receiving feed containing the additive.

- Crushed dried herbs and spices[6]

Most of the herbs and spices are consumed by humans in appreciable quantities and, their safety does not need further assessment, except for some that the Panel assessed.

3.2.2.1. Conclusions on the safety for the consumer

Considering the composition of BIOSTRONG® 510, the consumer exposure to any possible residues of the components of essential oils, quillaja bark, and the herbs and spices would be within the range of exposures considered safe for food use. The FEEDAP Panel, therefore, concludes that the use of BIOSTRONG® 510 as an additive in the feed for target animals would not measurably increase the exposure of consumers and, therefore, would not present a risk for the consumer.

3.2.3. Safety for the user[6]

- Effects on skin and eyes

No data on effects of BIOSTRONG® 510 on skin and eyes were provided by the applicant. The additive contains compounds with the potential to irritate mucous membranes (saponins from quillaja bark). Moreover, essential oils and crushed dried herbs present in BIOSTRONG® 510 may induce allergies in sensitive persons, handling the additive as recognised by the applicant in the Material Safety Data Sheet (MSDS).[24] Therefore, the FEEDAP Panel considers BIOSTRONG® 510 as an irritant to skin and eyes and as a skin sensitiser.

[23] Chronic study (2 years) in rat; dose: 0, 0.25, 0.5, 1% [c], conversions were calculated using standard values.
[24] Technical Dossier/Supplementary information July 2015/Annex II.5.2.1_updated.

- Effects on respiratory system

Exposure of the respiratory system of users handling BIOSTRONG® 510 can occur by inhalation of volatile components as well as dust of the additive. Data on particle size indicate that approximately 50% of the particles have a diameter below 100 μm and approximately 12% below 10 μm. The dusting potential of the additive according to Stauber–Heubach is up to 3.4 g/m^3, which suggest a high possibility of exposure during handling. Thus, it is likely that the respiratory tract of users is exposed to the dust. The additive contains components with a potential to irritate mucous membranes of the respiratory tract and to cause inflammation of lung tissue (saponins, silicon dioxide).

BIOSTRONG® 510 also contains a variety of components with the potential to induce allergic reactions (dried crushed herbs, essential oils). This assumption is confirmed by experience of the applicant, who classifies BIOSTRONG® 510 as a respiratory sensitiser on the basis of manufacturing experience.

3.2.3.1. Conclusions on the safety for the user

BIOSTRONG® 510 is considered as irritating to skin, eyes and the respiratory system, and as a potential skin and respiratory sensitiser.

3.2.4. Safety for the environment[6]

The additive contains compounds naturally present in the environment that will not result in a substantial increase in their concentration in the environment at the application rate of 150 mg BIOSTRONG® 510/kg feed for chickens for fattening. An environmental risk assessment of the major (thymol and *trans*-anethole) and other components of essential oils (linalool, *p*-cymene, γ-terpinene, carvacrol, β-myrcene, terpinen-4-ol, α-terpineol and anisaldehyde) showed no risk for the environment at the concentrations which were considered safe for targets species. The maximum concentration of saponins in BIOSTRONG® 510 results in 3.3 mg/kg feed. The corresponding predicted environmental concentrations in soil would be 17 μg saponin/kg when calculated for chickens for fattening according to the EFSA guidance (EFSA, 2008a). This low predicted environmental concentration can easily be surpassed by the growth of soapwort (*Saponaria officinalis*). Therefore, the use of BIOSTRONG® 510 at the recommended levels is not considered to be a risk for the environment.

3.3. Efficacy

The FEEDAP Panel considers that the studies made with the original formulation can be used to assess the efficacy of the new formulation.

The applicant submitted five long-term feeding studies supported by six short-term digestibility studies to demonstrate the efficacy of the additive in chickens for fattening. The mortality registered in one of the long-term studies[25] was high (14% on average) and, therefore, this study was not further considered in the assessment.

3.3.1. Long-term efficacy studies

Details on the design and the results of the four long-term studies[26] considered are presented in Table 3. Chickens were fed the experimental diets for 42 days in three studies and 35 days in another study. Trials 1, 2 and 3 were carried out as a 2 × 2 design, with two basal diets that differed in the nutrient and energy content and two levels of additive supplementation (non-supplemented vs supplementation at the recommended dose of 150 mg/kg feed). In the remaining trial (trial 4), two experimental diets were considered, a control diet and the control diet supplemented with the additive at the recommended dose. The basal diets (starter and grower) were based on maize, wheat and soya bean meal (in trial 1 wheat was not included) and were offered *ad libitum* in mash (studies 1, 2 and 3) or pelleted form (study 4). Confirmation of dose was obtained from the analysed thymol content of the diet. General conditions of the animals and mortality were checked/recorded. Feed intake and body weight were measured throughout the experimental period. Feed to gain ratio was calculated. In each study, an ANOVA was performed with the data considering the pen as the experimental unit. Differences were considered significant at a level of at least $p < 0.05$.

[25] Technical Dossier/Section IV/Annex IV.3.6. Conf.
[26] Technical Dossier/Section IV/Annex IV.3.7.Conf., Annex IV.3.8.Conf., Annex IV.3.9.Conf. and Annex IV.3.10.Conf.

Table 3: Effect of BIOSTRONG® 510 (150 mg/kg diet) on mortality and performance of chickens for fattening in four long-term studies

Study	(Sex, breed) Animals per replicate Replicates per treatment	Duration of the study (days)	BIOSTRONG® 510 (mg/kg feed)	Feed intake (g/day)	Final body weight (g)	Weight gain (g/day)	Feed to gain	Mortality (%)
1[(b)]	(♂, Ross 308) 30	35	0	90.5	1,689	47.1	1.92	7.1
	7		150	89.7	1,726	48.1	1.86[(a)]	5.2
2	(♂,♀; Ross 308) 22	42	0	111	2,417	56.5	1.96	6.8
	12		150	110	2,453	57.4	1.92	7.8
3	(♂, Cobb 500) 20	42	0	91.3	2,718	63.8	1.43	1.2
	6		150	88.9	2,744	64.4	1.38[(a)]	0.8
4	(♂, Cobb 500) 24	42	0	106.1	2,946	69.2	1.53	0.7
	6		150	103.9	2,997	70.4	1.48[(a)]	1.4

(a): Means of the treated group and controls within the same study are significantly different ($p < 0.05$).
(b): Technical Dossier/Section IV/Annex IV.3.7.Conf.

Mortality did not significantly differ between the groups in each study. The statistical analysis of the three studies, which included two dietary formulations, showed no interaction between the two main factors (type of diet and the addition of the additive). Therefore, only the results of the additive supplementation are presented. The supplementation of the diets with the additive resulted in a better feed to gain ratio in three studies (studies 1, 3 and 4) and in a better daily weight gain in one study (study 2).

The applicant submitted a meta-analysis[27] of the long-term studies. However, this included the study discounted on the ground of high mortality and, therefore, the results of the meta-analysis are not considered.

3.3.2. Short-term digestibility studies

Six short-term efficacy and digestibility studies have been performed in two different locations.[28] All studies shared a common design in which an equal number of male broilers were allocated to two treatments, a control, receiving a basal maize–soybean mean diet and the treated group, receiving the basal diet supplemented with BIOSTRONG® 510 at 150 mg/kg feed. Birds were fed *ad libitum* for 21 days. The diets contained an indigestible marker (titanium dioxide at 3 g/kg or chromium (III) oxide at 5 g/kg). Zootechnical performance (mortality, average daily feed intake, body weight, average daily gain and feed to gain ratio) was recorded for the experimental period of 1–21 days of age. At 21 days of age, all birds were killed and the ileum contents collected for analysis.

The addition of BIOSTRONG® 510 resulted in a significant improvement of digestibility of dry matter (1/6 studies), organic matter (3/6 studies), crude protein (4/6 studies) and crude fat (2/6 studies). Mortality and performance of chickens over the 21-day experimental period were as expected.

3.3.3. Conclusions on efficacy

BIOSTRONG® 510 has the potential to improve the performance of chickens for fattening by improving the feed to gain ratio or weight gain at a dose of 150 mg/kg complete feed. The results of the digestibility studies which indicated improved ileal digestibility is consistent with the effects seen in the long-term efficacy studies.

[27] Technical Dossier/ Section IV/Annex IV. 3.12.Conf.
[28] Technical Dossier/ Section IV/Annex IV. 3.1.Conf., Annex IV. 3.2.Conf., Annex IV. 3.3.Conf., Annex IV. 3.4.Conf., Annex IV. 3.5.Conf., and Annex IV. 3.10.Conf.

The FEEDAP Panel considers that these conclusions can be extended to chickens reared for laying and extrapolated to all minor poultry species for fattening or reared to point of lay at the same dose.

3.4. Post-market monitoring

The FEEDAP Panel considers 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.

4. Conclusions

The FEEDAP Panel concludes that BIOSTRONG® 510 is safe for chickens for fattening at the proposed dose of 150 mg/kg complete feed. The Panel considers that this conclusion can be extended to chickens reared for laying and extrapolated to all minor poultry species for fattening and reared to point of lay.

The FEEDAP Panel concludes that the use of BIOSTRONG® 510 as an additive in the feed for target species does not present risk for the consumer.

BIOSTRONG® 510 is considered as irritating to skin, eyes and the respiratory system, and as a potential skin and respiratory sensitiser.

The use of BIOSTRONG® 510 at the recommended dose is not considered to be a risk for the environment.

BIOSTRONG® 510 has the potential to improve the performance of chickens for fattening by improving the feed to gain ratio at a dose of 150 mg/kg complete feed. The FEEDAP Panel considers that this conclusion can be extended to chickens reared for laying and extrapolated to all minor poultry species for fattening and reared to point of lay at the same dose.

Documentation provided to EFSA

1) Dossier BIOSTRONG® 510. October 2011. Submitted by Delacon Biotechnik GmbH.
2) Dossier BIOSTRONG® 510. Supplementary information. May 2012. Submitted by Delacon Biotechnik GmbH.
3) Dossier BIOSTRONG® 510. Supplementary information. July 2012. Submitted by Delacon Biotechnik GmbH.
4) Dossier BIOSTRONG® 510. Supplementary information. May 2013. Submitted by Delacon Biotechnik GmbH.
5) Dossier BIOSTRONG® 510. Supplementary information. November 2014. Submitted by Delacon Biotechnik GmbH.
6) Dossier BIOSTRONG® 510. Supplementary information. July 2015. Submitted by Delacon Biotechnik GmbH.
7) Comments from Member States.

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[29] Regulation (EC) No 183/2005 of the European Parliament and of the Council of 12 January 2005 laying down requirements for feed hygiene. OJ L 35, 8.2.2005, p. 1.

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Abbreviations

ADI	acceptable daily intake
AFC	EFSA Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food
ANOVA	analysis of variance
bw	body weight
CEF Panel	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CFU	colony forming unit
EURL	European Union Reference Laboratory
FAO	Food Agricultural Organization
FEEDAP Panel	EFSA Panel on Additives and Products or Substances used in Animal Feed
FGE	food group evaluation
FLAVIS	The EU Flavour Information System
FL-no	FLAVIS number
GC–MS	gas chromatography–mass spectrometry
LOD	limit of detection
LOQ	limit of quantification
MCH	mean corpuscular haemoglobin
MCV	mean corpuscular volume
MCHC	mean corpuscular haemoglobin concentration
MSDS	Material Safety Data Sheet
PCDD	polychlorinated dibenzo-*p*-dioxin
PCDD/F	polychlorinated dibenzofuran
PhEur	European Pharmacopoeia
R_{rec}	recovery rate
RH	relative humidity
RSD_r	standard deviation for repeatability
RSD_{ip}	standard deviation for intermediate precision
TTC	threshold of toxicological concern
WHO	World Health Organization

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory (EURL) for Feed Additives on the Method(s) of Analysis for BIOSTRONG® 510

In the current application, authorisation is sought under Article 4(1) for BIOSTRONG® 510 under the category/functional group 4(a)&(d) 'zootechnical additives/digestibility enhancers & other zootechnical additives' according to Annex I of Regulation (EC) No 1831/2003. Authorisation is sought for the use of the *feed additive* for chickens and minor avian for fattening or rearing to point of lay. BIOSTRONG® 510 is a preparation of partially microencapsulated essential oils of thyme and star anise, with a guaranteed minimum content of the active substance (*Thymol*) of 2 g/kg in an excipient based on mixed dried herbs and spices, and other bulking and anticaking agents. The feed additive is intended to be incorporated in complete or complementary *feedingstuffs* through *premixtures*. The Applicant proposed a dosage of 150 mg BIOSTRONG® 510/kg *feedingstuffs*, which corresponds to 0.3 mg/kg of *Thymol* in *feedingstuffs*.

For the determination of *Thymol* in the *feed additive*, *premixtures* and *feedingstuffs*, the Applicant proposed a single-laboratory validated and further verified method based on gas chromatography–mass spectrometry (GC–MS). The following performance characteristics were reported:

- a standard deviation for *repeatability* (RSD_r) and for *intermediate precision* (RSD_{ip}) ranging from 1.4 to 9.2%;
- a *recovery rate* (R_{rec}) ranging from 98.3 to 119%;
- a limit of *quantification* (LOQ) of 21 μg/kg.

Based on these performance characteristics, the EURL recommends for official control the single-laboratory validated and further verified method based on gas chromatography–mass spectrometry (GC–MS) for the determination of *Thymol* in the *feed additive*, *premixtures* and *feedingstuffs*.

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 10 (Commission Regulation (EC) No 378/2005) is not considered necessary.