SCIENTIFIC OPINION

Scientific Opinion on Safety and efficacy of AviPlus® as feed additive for weaned piglets¹

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)²,³

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

AviPlus® is based on a mixture of citric (25%) and sorbic (16.7%) acids, thymol (1.7%) and vanillin (1.0%). It is intended for use with weaned piglets at 1–3 g/kg complete feed. The applicant initially did not consider vanillin to be an integral component of AviPlus® and so did not monitor its concentration. Thus, it is assumed that vanillin was present at the specified concentration in the test substance used in the studies described. In the absence of any adverse effects at ten times the maximum recommended dose, the FEEDAP Panel considers that AviPlus® is safe for weaned piglets when used at the dose range proposed. As all the active components in AviPlus® occur naturally and are authorised for use in human food, the FEEDAP Panel considers that the use of the additive in animal feed is safe for consumers. The potential for irritation/sensitisation by dermal/ocular exposure cannot be fully excluded. No environmental assessment is considered necessary as the active components occur widely in nature and the use of the additive would not detectably add to the existing environmental load. Seven studies were provided to establish efficacy, three with the maximum and four the minimum recommended dose. Significant improvements in daily weight gain and feed to gain ratio were seen in all three studies with the maximum recommended dose. Benefits were seen at the minimum dose in only two of the four studies but a meta-analysis of the four studies showed an overall significant improvement in feed to gain ratio and a higher final bodyweight. Consequently, the FEEDAP Panel concludes that AviPlus® has the potential to increase the growth rate of piglets and improve feed to gain ratio with the minimum recommended dose.

KEY WORDS

Zootechnical additive, other zootechnical additives, citric acid, sorbic acid, thymol, vanillin, piglets, safety, efficacy

¹ On request form the European Commission, Question No EFSA-Q-2008-701, adopted on 25 May 2010.
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³ Acknowledgement: The Panel wishes to thank the members of the Working Group on Organic acids, including Atte Von Wright and Pieter Wester for the preparation of this opinion.

SUMMARY

Following a request from the European Commission, the European Food Safety Authority was asked to deliver a scientific opinion on the safety and efficacy of AviPlus® as feed additive for weaned piglets.

AviPlus® is the trade name for a feed additive based on two organic acids (citric and sorbic acids), the monoterpenes thymol and vanillin. It is intended for use with weaned piglets at doses between 1–3 g/kg complete feed (equivalent to a maximum of 750 mg citric acid, 500 mg sorbic acid, 51 mg thymol and 30 mg vanillin/kg feed) to improve growth rate and/or efficiency of feed conversion.

The applicant initially did not consider vanillin to be an integral component of AviPlus®, although it was blended with the other components, and consequently did not monitor its concentration. In addition, the stability of vanillin in the presence of the other components of the additive during storage and under use conditions has not been established. As a result, it can only be assumed that vanillin was present at the specified concentration in the various batches of the test substance used in the studies described.

In the absence of any adverse effects in piglets given AviPlus® at ten times the maximum recommended dose, the FEEDAP Panel considers that the additive is safe for weaned piglets when used at the dose range proposed by the applicant.

All four of the active substances which constitute AviPlus® occur naturally and/or are already authorised for use in human food. The FEEDAP Panel considers that the use of the additive in animal feed at concentrations lower than that authorised for foods will not detectably alter consumer exposure to the active components (or their residues). Consequently, the use of the additive under the conditions proposed is safe for consumers.

Because of the large particle size of the product, its use is unlikely to represent a respiratory hazard but the potential for irritation/sensitisation associated with dermal (and ocular) exposure cannot be fully excluded. Therefore, the FEEDAP Panel would support the use of the protective measures recommended by the applicant.

Since the active components all occur widely in nature and as the use of the additive would not detectably add to the existing environmental load, no further environmental assessment is considered necessary.

Significant improvements in daily weight gain and feed to gain ratio were seen in all three studies in which the additive was included at the maximum recommended dose. Benefits were seen at the minimum dose in only two out of four studies, but a meta-analysis of the four feeding studies showed an overall significant improvement in feed to gain ratio and a higher final bodyweight. Consequently, the FEEDAP Panel concludes that AviPlus® has the potential to increase the growth rate of piglets and improve feed to gain ratio with the minimum recommended dose. The use of the additive at the proposed dose range is considered unlikely to adversely affect product quality.
# TABLE OF CONTENTS

Abstract ................................................................. 1  
Key words ......................................................................................... 1  
Summary .......................................................................................... 2  
Table of contents ............................................................................. 3  
Background ....................................................................................... 4  
Terms of reference ............................................................................ 4  
Assessment ......................................................................................... 6  

1. Introduction .................................................................................. 6  
2. Characterisation of the product .................................................. 6  
   2.1. Composition and production of the additive ....................... 6  
   2.2. Characterisation of the active substances ......................... 7  
   2.3. Stability and homogeneity ................................................... 7  
      2.3.1. Incompatibilities ............................................................ 7  
      2.3.2. Conditions of use ......................................................... 8  
      2.3.3. Evaluation of the analytical methods by the Community Reference Laboratory (CRL) 8  
3. Safety .......................................................................................... 8  
   3.1. Studies concerning the safety of use of the additive for the target animals ................. 8  
      3.1.1. Tolerance studies for weaned piglets ......................... 8  
      3.1.2. Microbial studies ......................................................... 9  
      3.1.3. Conclusions on the safety for piglets ....................... 9  
   3.2. Safety for the consumer ...................................................... 9  
   3.3. Safety for the user ............................................................. 9  
   3.4 Safety for the environment ............................................... 9  
4. Efficacy ..................................................................................... 10  
   4.1. Effect on the quality of animal products ......................... 11  
5. Post-market monitoring .............................................................. 12  
Conclusions and Recommendations .............................................. 12  
Documentation provided to EFSA ............................................... 12  
References ..................................................................................... 13  
Appendix ....................................................................................... 14
BACKGROUND

Regulation (EC) No 1831/2003\(^4\) 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 Vetagro S.P.A.\(^5\) for authorisation of the product AviPlus\(^6\), a preparation of citric acid, sorbic acid, thymol, to be used as a feed additive for weaned piglets (category: zootechnical additive; functional group: other zootechnical additive) under the conditions proposed 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.\(^6\) 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 6 May 2009.

The additive is not authorised in the EU.

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), consumer and user and the environment of the product AviPlus\(^6\), a preparation of citric acid, sorbic acid and thymol, when used under the conditions described in Table 1.

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\(^4\) OJ L 268, 18.10.2003, p. 29.
\(^5\) Via Porro 2 - 42124 Reggio Emilia – Italy.
\(^6\) EFSA Dossier reference: FAD-2008-0049.
Table 1: Description and conditions of use of the additive as proposed by the applicant

<table>
<thead>
<tr>
<th>Additive</th>
<th>AviPlus®</th>
</tr>
</thead>
<tbody>
<tr>
<td>Registration number/EC No/No (if appropriate)</td>
<td>4d xxx</td>
</tr>
<tr>
<td>Category(ies) of additive</td>
<td>Zootechnical feed additive</td>
</tr>
<tr>
<td>Functional group(s) of additive</td>
<td>Other zootechnical additives</td>
</tr>
</tbody>
</table>

**Description**

<table>
<thead>
<tr>
<th>Composition, description</th>
<th>Chemical formula</th>
<th>Purity criteria (if appropriate)</th>
<th>Method of analysis (if appropriate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preparation of protected microbeads containing citric acid, sorbic acid, thymol</td>
<td>Citric acid 25% Sorbic acid 16.7% Thymol 1.7%</td>
<td>Complies with EU law on undesirable substances</td>
<td>Validated HPLC methods</td>
</tr>
</tbody>
</table>

**Trade name (if appropriate)**

<table>
<thead>
<tr>
<th>AviPlus®</th>
</tr>
</thead>
</table>

**Name of the holder of authorisation (if appropriate)**

<table>
<thead>
<tr>
<th>Vetagro S.P.A.</th>
</tr>
</thead>
</table>

**Conditions of use**

<table>
<thead>
<tr>
<th>Species or category of animal</th>
<th>Maximum Age</th>
<th>Minimum content</th>
<th>Maximum content</th>
<th>Withdrawal period (if appropriate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weaned piglets</td>
<td>-</td>
<td>1000 mg/kg</td>
<td>3000 mg/kg</td>
<td>Not relevant</td>
</tr>
</tbody>
</table>

**Other provisions and additional requirements for the labelling**

<table>
<thead>
<tr>
<th>Specific conditions or restrictions for use (if appropriate)</th>
<th>Store in original, closed packaging, in cool, dry place</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific conditions or restrictions for handling (if appropriate)</td>
<td>For user safety, breathing protection and safety glasses</td>
</tr>
<tr>
<td>Post market monitoring (if appropriate)</td>
<td>Vetagro will conduct post-marketing monitoring in compliance with EU law on feed hygiene, namely by use of HACCP and traceability systems, and formal monitoring of customer feedback through product or service complaints</td>
</tr>
<tr>
<td>Specific conditions for use in complementary feedingstuffs (if appropriate)</td>
<td>Dosage should supply 1000-3000 mg/kg in final feed</td>
</tr>
</tbody>
</table>

**Maximum Residue Limit (MRL) (if appropriate)**

<table>
<thead>
<tr>
<th>Marker residue</th>
<th>Species or category of animal</th>
<th>Target tissue(s) or food products</th>
<th>Maximum content in tissues</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not relevant</td>
<td>Not relevant</td>
<td>Not relevant</td>
<td>Not relevant</td>
</tr>
</tbody>
</table>
**ASSESSMENT**

1. **Introduction**

AviPlus® is the trade name for a feed additive based on two organic acids (citric and sorbic acids), the monoterpenic thymol and vanillin. It is intended for use with weaned piglets to improve growth rate and/or efficiency of feed conversion. This zootechnical effect is claimed by the applicant to be due to a potential of the additive to stimulate feed intake (a flavour/appetent effect) and to favourably ‘regulate’ the gut microbiota.

Although the additive itself has not previously been authorised for use in the EU, the declared active components are widely used in feed and food for a variety of purposes. Citric and sorbic acids are currently approved in the EU as technological feed additives for all animal categories with no minimum or maximum concentration specified for final feeds. Both acids are included in the functional group preservatives and citric acid is also approved for use as a binder, coagulant and anticaking agent. The acids are also permitted food additives (Directive EC 95/2/1995); citric acid (E330) at concentrations up to 5000 mg/kg and sorbic acid (E200) at concentrations up to 2000 mg/kg. The third active component thymol is also authorised in the EU for use in feeds as a sensory additive and as a food flavour (Commission Decision of 23 January 2002 amending Commission Decision 1999/217/EC). Vanillin, the fourth component, is currently authorised as a sensory feed additive without limitation and is also widely used to add vanilla flavour or aroma to human foods.

2. **Characterisation of the product**

2.1. **Composition and production of the additive**

The additive is specified to contain 250 g/kg citric acid, 167 g/kg sorbic acid, 17 g/kg thymol and 10 g/kg vanillin. The analysis of six batches of AviPlus® showed that this specification was met (CV % < 5.0) with respect to the first three active substances. No analyses have been performed to confirm the presence of vanillin. In addition to the active substances, the additive also contains hydrogenated triglycerides of vegetable origin (498 g/kg), lecithin as emulsifier (20 g/kg) and silicon dioxide as an additional anticaking agent (38 g/kg). The excipients are all of food-grade standard with a guaranteed purity of > 99.5 % (confirmed by certificates of analysis).

The applicant considers that the function of vanillin is simply to add aroma, and as it is an already authorised sensory additive they did not initially consider it to be an integral component of AviPlus®, although it was blended with the other components. They sought to retain a degree of flexibility by describing this additional sensory component as ‘vanillin or other EU-approved flavour’. However, in the view of the FEEDAP Panel, vanillin should be considered as an active component since it possesses biological activities potentially relevant to the use of AviPlus® as a zootechnical additive and, as such, should be included in the product specification. The applicant has accepted this and now formally considers vanillin to be an active component of AviPlus®. However, as a consequence of their earlier view, although vanillin was included in all batches of the additive used in the studies reported it was not included in any monitoring of the active components of the additive.

The additive is manufactured by mixing the active substances with the heated vegetable oil and the other components. The mixture is then spray-chilled under pressure. The resulting additive is in the form of fat-coated spherical microbeads with diameters ranging from 400 to 2000 μm. Particle size analysis of six batches of the additive showed that five batches contained no detectable particles with a diameter < 400 μm, while a sixth lot had only 0.01 % of particles below 400 μm.

Data on undesirable substances as possible contaminants (heavy metals, arsenic, fluorine, dioxins and dioxin-like PCBs and aflatoxin B1) was provided for six different batches of the additive. In all cases, the values were below the maximum permitted level in feed materials. Aerobic and anaerobic plate counts and counts of total coliforms, *Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus*...
*aureus*, filamentous fungi and yeast were < 10 CFU/g product (< 3 MPN/g product in the case of coliforms) in six batches of the additive. No salmonella were detected in 25 g of product.

### 2.2. Characterisation of the active substances

Citric acid (2-hydroxy-1,2,3-propanetricarboxylic acid, CAS Nº 77-92-9 anhydrous) is produced by fermentation of a non-GMO strain of *Aspergillus niger* (ATCC 26550). The citric acid is recovered from the fermentation medium by adsorption, the citric acid solution is then purified and crystallised. Certificates of analysis of two batches provided by the manufacturer show a purity > 99.5%.

Sorbic acid (2,4-hexadienoic acid, CAS Nº 110-44-1) is produced by chemical synthesis and purified to food grade standards. Again, certificates of analysis of two batches provided by the manufacturer showed a purity > 99.5%.

Thymol (5-methyl-2-(1-methylethyl)phenol, Flavis Nº 04.006, CAS Nº 89-83-8) is also produced synthetically by a condensation of *m*-cresol and propene, followed by alkylation, purification by distillation and crystallisation. Purity is given by the applicant as > 98%, although the single certificate of analysis provided by the manufacturer was higher than 99% by weight.

Vanillin (4-hydroxy-3-methoxybenzaldehyde, Flavis Nº 05.018, CAS Nº 121-33-5) is produced by synthesis from guaiacol. Certificates of analysis of two batches provided by the supplier showed that the purity was higher than 99.5% by weight.

### 2.3. Stability and homogeneity

The stability of the additive (three batches) was studied under two simulated storage conditions, 25 °C/60% RH and 40 °C/75% RH, in typical packaging. At 25 °C, both acids showed good stability with a recovery of > 92% after 18 months of storage. As could be expected, thymol was more susceptible to deterioration with losses of approximately 28% after 12 months and 35% after 18 months of storage. At the higher temperature, both acids were stable for six months (the maximum time tested) but 29% of the thymol was lost to the analysis.

A similar stability pattern was seen in premixtures and in feedingstuffs stored at 25 °C. In two batches (three replicates of each batch) of a vitamin-mineral premix there was little loss of the acids after four months of storage but losses of thymol over the same time period ranged from 13 to 30% (mean 22%). In comparable experiments made with mash and pelleted feed, the acids were stable in feed for at least three months but the mean loss of thymol was 35.2% of the initial analysis in the mash feed after three months. Thymol showed better stability in pelleted feed where the mean loss was 24%. Thymol was also more sensitive to the pelleting process with approximately one third lost at 65 °C. Pelleting at higher temperatures was not described but, on the basis of the result at 65 °C, this would appear inadvisable.

The homogenous distribution of AviPlus® was studied in a complete feed and a mineral-vitamin premix for piglets (ten samples each). The measured mean homogeneity (100-CV) in complete feeds was 94%, 96% and 85% for thymol, sorbic acid and citric acid, respectively. Citric acid was determined as the difference between the total citric acid in the supplemented feed and the citric acid naturally occurring in the unsupplemented feed. This may explain the apparently poorer distribution of this component of the mixture. In premixtures, the mean homogeneity was 97%, 96% and 98% for thymol, sorbic acid and citric acid, respectively. In this case, the citric acid was distributed in line with the other components supporting the view that the result in the complete feed was anomalous and due to analytical problems.

The applicant declined to provide the requested studies on the stability and homogeneous distribution of vanillin in the additive, in premixtures and in feedingstuffs.

### 2.3.1. Incompatibilities

No incompatibilities or adverse interactions with feed components, carriers, other approved additives or medical products are to be expected.
2.3.2. **Conditions of use**

AviPlus® is intended for use in feeds for weaned piglets with a dose range of 1000–3000 mg/kg complete feedingstuffs (equivalent to a maximum of 750 mg citric acid, 500 mg sorbic acid, 51 mg thymol and 30 mg vanillin/kg feed). No withdrawal period is proposed.

2.3.3. **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 substances in animal feed. The Executive Summary of the CRL report, which deals with the methods for citric and sorbic acids and thymol, can be found in the appendix. Since vanillin has been included in the product specification, the applicant was asked to provide analytical methods for the analysis of vanillin in the additive, in premixtures and in feedingstuffs. The applicant declined to provide the methods, therefore the CRL could not evaluate the methods for vanillin.

3. **Safety**

3.1. **Studies concerning the safety of use of the additive for the target animals**

3.1.1. **Tolerance studies for weaned piglets**

A 42-day tolerance study was carried out with 144 weaned piglets weighing 6.7 (±0.84) kg at the start of the study. A group fed unsupplemented feed was compared with treatment groups given the basal diet supplemented with the maximum recommended dose (3 g/kg feed), a 3.3–fold overdose (10 g/kg) and a tenfold overdose (30 g/kg) of the additive. The expected concentration of the additive in the feed was confirmed by analysis of three of the four components. Each treatment was replicated in nine pens with four animals per pen (five with castrated males and four with females). Routine zootechnical parameters were monitored and animals inspected daily for clinical symptoms. Blood samples were taken from seven pigs per treatment at day 0 and then six pigs per treatment (three per sex) on day 42 for routine haematology and clinical chemistry. The animals sampled at day 42 were killed for necropsy. Urine samples were obtained from the sacrificed animals. Faecal samples were collected at day 35 from ten animals from the control and the maximum recommended dose groups and were analysed for lactobacilli, coliforms and clostridia counts.

Mortality during the trial was of 9 % but there was no significant difference in morbidity or mortality between treatment groups. Mortality was ascribed principally to gastric ulceration as a consequence of the low particle size of the experimental feed (mash), and to *E. coli* infections arising after the start of the study. All piglets on trial were treated once with a single injection of enrofloxacin to prevent post-weaning *E. coli* diarrhoea.

The zootechnical parameters were not significantly affected by the supplementation with AviPlus® at any concentration (control: ADG 316 g/d, feed to gain ratio: 1.65). No evidence of adverse effects of treatment were seen in any of the necropsy results or in the results from the urine analysis. Significantly elevated values for blood triglycerides were seen in two of the three supplemented groups compared to the control group, but this was not obviously treatment-related and all the values remained within the expected physiological range (lowest in control (0.6 mmol/l) and highest in the maximum recommended dose group (1.62 mmol/l)). The percentage of basophils was also significantly increased in the highest supplemented group but at 0.82 % also remained within the physiological range. Other blood chemistry and haematology values were not affected by the treatments.
3.1.2. Microbial studies

As indicated above, faecal samples were collected from piglets in the control and the highest dose groups and examined for a limited number of microbial species. No significant differences in counts of culturable lactobacilli, coliforms or clostridia were detected.

3.1.3. Conclusions on the safety for piglets

The FEEDAP Panel concludes that in the absence of any adverse effects in piglets given AviPlus® at ten times the maximum recommended dose, the additive is safe when used at the dose range proposed by the applicant.

3.2. Safety for the consumer

Citric and sorbic acids are authorised for use in food in concentrations greater (5000 and 2000 mg/kg, respectively) than that proposed by the applicant for use in complete feedingstuffs. Both acids are extensively metabolised by well-understood routes and neither will lead to residues of any relevance to consumer safety.

EMEA (1996), echoing an earlier report by the Council of Europe, noted that thymol was a natural component of the human diet and widely used as a food additive at concentration up to 78 mg/kg. As the compound is rapidly metabolised and eliminated via urine, they concluded that residues of thymol in animal products were unlikely to be of any toxicological concern and did not require an MRL to be set.

Vanillin or its acid are naturally present in most plants used for food or feeds. It is not considered by JECFA (1967) to raise safety concerns at the concentrations used for flavouring purposes. The 57th meeting of JECFA (2001) retained the ADI of 10 mg/kg body weight. Vanillin is readily oxidised in the liver and excreted via urine as the free acid or its conjugates.

The FEEDAP Panel concludes that the use of the additive in animal feed will not detectably alter consumer exposure to the active components of the additive or to residues of concern in the products of animals given the additive.

3.3. Safety for the user

No specific studies for the safety for the user have been provided.

All four components individually are known to irritate skin and eyes and are labelled accordingly. However, this product is fat coated, which would be expected to substantially reduce user exposure to the active agents.

The lowest particle size for the product was demonstrated to be 400 µm and so the potential for respiratory toxicity can be reasonably considered as absent. No data on skin sensitisation is provided although it should be noted that thymol has extensive cosmetic and dental applications.

In the absence of specific studies to assess the level of risk to users of irritation/sensitisation via dermal or ocular routes, the applicant recommends the use of various barrier methods.

The FEEDAP Panel concludes based on particle size that the use of the additive is unlikely to represent a respiratory hazard but the potential for irritation/sensitisation associated with dermal (and ocular) exposure cannot be fully excluded.

3.4 Safety for the environment

Since the active components all occur widely in nature and as the use of the additive would not add to the present environmental load, no further environmental assessment is considered necessary.
4. Efficacy

Seven studies performed with the product AviPlus® on weaned piglets in three European countries were described. However, it should be noted that five of the seven studies were made at the same centre weighting the results towards the production conditions typical of a particular region. For all the seven studies, only concentrations of citric acid, sorbic acid and thymol were confirmed by analysis of the treated feed. Analysis on the concentrations of vanillin have not been performed.

The studies had very similar designs, in which the performance of a group of animals given a basal diet based on cereal and soybean was compared to a second group given the same basal diet supplemented with AviPlus® at either the minimum or the maximum recommended level. No other growth promoters were used. The parameters measured were feed intake, daily weight gain, feed to gain ratio and mortality. Any treatments for diarrhoea and the total dead/culled ratio were recorded. All data were statistically analysed by ANOVA.

The first efficacy trial differed from the other studies with regard to duration and measure of feed intake and is separately reported (Table 2). In this study, 256 weaned pigs (mean weight 7.6 kg, unspecified breed) were assigned to each treatment and housed in 38 pens each containing six to seven animals. The duration of the study was 49 days. There were three treatments in total: an unsupplemented control group, a group supplemented with 3 g/kg feed AviPlus® and a third group involving another additive. The results from this third group are disregarded. Feed intake was reported as Feed Units for growing pigs (FUgp).

Supplementation of the diet with AviPlus® at the maximum recommended dose significantly increased weight gain and feed to gain ratio of piglets without any significant effect on feed intake (Table 3).

Table 2: Effect of AviPlus® on the performance of weaned piglets (first study)

<table>
<thead>
<tr>
<th>AviPlus® (g/kg)</th>
<th>Daily feed intake (FUgp/pig)</th>
<th>Weight gain (g/day)</th>
<th>Feed/gain (FUgp/kg gain)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.79</td>
<td>437(^a)</td>
<td>1.81(^a)</td>
</tr>
<tr>
<td>3</td>
<td>0.81</td>
<td>462(^b)</td>
<td>1.76(^b)</td>
</tr>
</tbody>
</table>

FUgp = Feed Units for growing pigs
\(^a\, ^b\) Values in a column with no common superscript are significantly different (P < 0.05)

The next five studies, all made at the same centre, lasted for 42 days and involved in each case a total of 144 piglets and two treatments. The animals on each treatment (72) were housed in 18 pens with four piglets per pen. The mean start weights of piglets for the five studies were 6.8, 7.1, 8.3, 6.2 and 7.9 kg, respectively. The breed selected for study differed between trials (Goland, Goland x PIC or MB1 LW B2). Feeds were based on wheat, barley and soybean and were fed as a mash.

Mortality was low in all studies with the exception of trial 4, where a respiratory disease due to secondary bacterial infections increased mortality to 11.1%. No veterinary treatment was administered during the trial as the clinical picture presented was considered relatively mild in most affected piglets.

The results of studies 2 to 6 are presented in Table 3. Overall, piglets receiving AviPlus® had a higher final weight or weight gain reaching significance in three trials, a significant lower feed/gain ratio in three trials and a trend to lower feed/gain in a fourth trial compared to those receiving the control diet.

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7 Technical dossier, Section IV, annex IV_3_1.
Table 3: Effect of AviPlus® on the performance of weaned piglets (studies two to six)

<table>
<thead>
<tr>
<th>Study</th>
<th>AviPlus® (g/kg)</th>
<th>Final weight (kg)</th>
<th>Feed intake (g/day)</th>
<th>Weight gain (g/day)</th>
<th>Feed to gain</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0</td>
<td>20.5</td>
<td>573</td>
<td>319</td>
<td>1.82</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>21.9*</td>
<td>557</td>
<td>347</td>
<td>1.62**</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>28.0</td>
<td>864</td>
<td>494</td>
<td>1.75</td>
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<tr>
<td></td>
<td>3</td>
<td>29.0**</td>
<td>857</td>
<td>516**</td>
<td>1.66*</td>
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<tr>
<td>4</td>
<td>0</td>
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<td>569</td>
<td>307</td>
<td>1.86</td>
</tr>
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<td></td>
<td>3</td>
<td>23.3*</td>
<td>561</td>
<td>334**</td>
<td>1.68</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>20.9</td>
<td>577</td>
<td>351</td>
<td>1.65</td>
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<tr>
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<td>20.9</td>
<td>573</td>
<td>350</td>
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<tr>
<td>6</td>
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<td>606</td>
<td>309</td>
<td>1.96</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>21.7</td>
<td>604</td>
<td>325*</td>
<td>1.87a</td>
</tr>
</tbody>
</table>

*,** Significantly different from control values at P < 0.05 or 0.01, respectively.
a P=0.0514

The final study (study 7) resembled the previous six but used a different feed (pelleted wheat-soybean) and a higher number of animals. A total of 288 pigs (Landrace x Duroc x PIC) were assigned either to a control group or to a treatment group receiving the minimum recommended dose. There were 24 replicated pens of six pigs per treatment. The duration of the experiment was 42 days. Mean values for weight gain, final weight (30.5 kg), feed intake and feed to gain ratio were virtually identical in the control and treatment groups.

A meta-analysis was made of the pooled results of the four studies (studies 2, 5, 6 and 7) in which the additive was used at the minimum recommended dose (1 g additive/kg complete feed). This showed significant effects of treatment on body weight (23.3 vs. 23.8 kg, P = 0.026), daily gain (381 vs. 392 g, P = 0.051) and feed to gain ratio (1.71 vs. 1.63, P = 0.0002).

4.1. Effect on the quality of animal products

The sensory property of meat from treated animals was not directly assessed. However, in the absence of residues from the organic acids and given the very limited residues arising from the thymol and vanillin, it is very unlikely that detectable differences in taste would arise.

4.3 Conclusion on efficacy

Significant improvements in daily weight gain and feed conversion were seen in all three studies in which the additive was included at the maximum recommended dose. However, efficacy at the minimum dose was seen in only two of the four studies and it required a meta-analysis to provide evidence of beneficial effects on feed to gain ratio and body weight gain at this dose. Consequently, the FEEDAP Panel concludes that there is evidence that AviPlus® has the potential to increase the growth rate of pigs and improve feed to gain ratio when used at the both the minimum and the maximum recommended dose with the greater effect seen with the higher dose. No significant effects on feed intake were evident.

It is considered unlikely that use of the product over the dose range proposed would adversely affect product quality.

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8 Technical dossier, Section IV, annex IV_3_2.
9 Technical dossier, Section IV, annex IV_3_3.
10 Technical dossier, Section IV, annex IV_3_4.
11 Technical dossier, Section IV, annex IV_3_5.
12 Technical dossier, Section IV, annex IV_3_6.
13 Technical dossier, Section IV, annex IV_3_7.
14 Technical dossier, Section IV, annex IV_3_8.
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\(^{15}\) and Good Manufacturing Practice.

**CONCLUSIONS AND RECOMMENDATIONS**

**CONCLUSIONS**

The stability of vanillin in the presence of the other components of the additive has not been established.

In the absence of any adverse effects in piglets given AviPlus\(^\circledR\) at ten times the maximum recommended dose, the additive is considered safe for weaned piglets when used at the dose range proposed by the applicant.

The use of the additive in animal feed will not detectably alter consumer exposure to the active components of the additive or to residues in the products of animals given the additive. Consequently, the use of the additive under the conditions proposed is safe for consumers.

The use of the additive is unlikely to represent a respiratory hazard but the potential for irritation/sensitisation associated with dermal (and ocular) exposure cannot be fully excluded. Therefore, the FEEDAP Panel would support the use of the protective measures recommended by the applicant.

Since the active components all occur widely in nature and as the use of the additive would not detectably add to the existing environmental load, no further environmental assessment is considered necessary.

The FEEDAP Panel concludes that there is evidence that AviPlus\(^\circledR\) has the potential to increase the growth rate of pigs and improve feed to gain ratio when used at both the minimum and the maximum recommended dose with the greater effect seen with the higher dose.

The use of the product over the dose range proposed is considered unlikely to adversely affect product quality.

**RECOMMENDATIONS**

The vanillin content (1.0%) should be included in the description of the product.

Analytical methods for vanillin suitable for control purposes are required.

**DOCUMENTATION PROVIDED TO EFSA**

1. AviPlus\(^\circledR\) (preparation of protected microbeads containing citric acid (25%), sorbic acid (16.7%) and thymol (1.7%)) for weaned piglets. September 2008. Submitted by Vetagro S.P.A.
2. Supplementary Information on AviPlus\(^\circledR\). October 2009. Submitted by Vetagro S.P.A.
3. Supplementary Information on AviPlus\(^\circledR\). March 2010. Submitted by Vetagro S.P.A.
4. Evaluation report of the Community Reference Laboratory for Feed Additives on the methods(s) of analysis for citric acid, sorbic acid and thymol.
5. Comments from Member States received through the ScienceNet.

REFERENCES


APPENDIX

Executive Summary of the Evaluation Report of the Community Reference Laboratory for Feed Additives on the Method(s) of Analysis for citric acid, sorbic acid and thymol

In the current application authorisation is sought for AviPlus according to Article 4 (1) of Regulation (EC) No 1831/2003 under the category "zootechnical additives", group 4(d) "other zootechnical additive", according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought to use AviPlus as additive improving growth and/or feed efficacy of weaned piglets. The additive is intended to be marketed in forms of micro beads, containing 25 % of citric acid, 16.7 % of sorbic acid and 1.7 % of thymol in matrix of saturated vegetable fats.

The active agents of AviPlus are citric acid (E 330), sorbic acid (E 200) and thymol (Flavis Nº 04.006), all approved additives.

The feed additive is intended to be incorporated into premixtures and/or complete feedingstuffs to obtain a recommended concentration ranging from 1000 to 3000 mg feed additive per kg of complete feedingstuffs for piglets. The corresponding concentration ranges in complete feedingstuffs for piglets are: from 250 to 750 mg/kg for citric acid, from 167 to 501 mg/kg for sorbic acid and from 17 mg/kg to 51 mg for thymol.

For the determination of the citric acid (E 330) in the feed additive (AviPlus) and premixtures a reverse phase high performance liquid chromatography method equipped with ultraviolet/diode array detection (RP-HPLC-UV/DAD) is proposed by the applicant. The following acceptable performance characteristics obtained from the in-house validation study were reported: - a limit of determination (LOD) of 5 mg/kg; - a limit of quantification (LOQ) of 10 mg/kg; - a recovery rate of 100 % determined at different concentration levels; - a repeatability relative standard deviation (RSDr) of 3.3 % for feed additive and 5.2 % for premixtures.

For the determination of citric acid (E 330) in the feedingstuffs the applicant proposes an enzymatic method, based on the CEN standardized method for the determination of citric acid in fruit and vegetable juices (EN 1137). The following acceptable performance characteristics obtained from the in-house validation study were reported: - LOD = 5 mg/kg; - LOQ = 10 mg/kg; - a recovery rate of 100 %; - RSDr = 1.6 %.

Samples of feed additive (AviPlus), premixtures and feedingstuffs were sent to a second independent laboratory for determination of citric acid. The reported results were in agreement with those obtained by the applicant, thus demonstrating the transferability of the applicant's method.

Based on these acceptable performance characteristics, the applicant in-house validated and verified methods for the determination of citric acid are recommended for official control purposes in the frame of authorisation.

For the determination of the sorbic acid (E 200) in the feed additive (AviPlus), premixtures and feedingstuffs the RP-HPLC-UV/DAD method is proposed by the applicant. The following acceptable performance characteristics obtained on the in-house validation were reported: LOD = 10 mg/kg; - LOQ = 25 mg/kg; - a recovery rate of 100 % for feed additive (AviPlus) and premixtures, 85 % for feedingstuffs; - RSDr = 2.9 % for feed additive, 4.1 % for premixtures and 4.2 % for feedingstuffs.

Samples of feed additive (AviPlus), premixtures and feedingstuffs were sent to a second independent laboratory for determination of sorbic acid. The reported results were in agreement with those obtained by the applicant, thus demonstrating the transferability of the applicant's method.

Based on these acceptable performance characteristics, the applicant in-house validated and validated method for the determination of sorbic acid is recommended for official control purposes in the frame of authorisation.
For the determination of the **thymol** in the *feed additive* (AviPlus), *premixtures* and *feedingstuffs* the RP-HPLC-UV/DAD method is proposed by the applicant. The following acceptable performance characteristics obtained on the in-house validation were reported: - LOQ = 2.5 mg/kg; - a recovery rate ranging from 90 to 100 % depending on matrix; - RSD, ranging from 2.9 to 3.4 %, for different matrixes.

Samples of *feed additive* (AviPlus), *premixtures* and *feedingstuffs* were sent to a second independent laboratory for determination of thymol. The reported results were in agreement with those obtained by the applicant, thus demonstrating the transferability of the applicant's method.

Based on these acceptable performance characteristics, the applicant in-house validated and verified method for the determination of thymol is recommended for official control purposes in the frame of authorisation.

Further testing or validation is not considered necessary.