ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES

DRAFT OPINION ON AN APPLICATION UNDER THE NOVEL FOODS REGULATION FOR ROOSTER COMBS EXTRACT

Applicant:	**Bioiberica S.A.**
Responsible Person:	**Laura Vicente**
EC Classification:	**2.1**

Introduction

1. An application was submitted to the Food Standards Agency in February 2011 by Bioiberica S.A. for the authorisation of rooster combs extract (RCE) as a novel ingredient in the EU. A copy of the application was placed on the Agency's website for public consultation.

2. Rooster combs have been widely consumed in Europe as part of traditional dishes. RCE is an extract rich (60-80%) in sodium hyaluronate (SH) which is found in the intracellular matrix of animal and human connective tissues e.g. rooster combs. The applicant states that SH helps in lubricating and cushioning joints.

3. In addition to SH, RCE also contains glycosaminoglycans (approx. 20%) and partially hydrolysed proteins (approx. 20 %). Glycosaminoglycans are long unbranched chains of polysaccharides made up of repeating disaccharide units. The hydrolysed proteins are polypeptides, peptides and amino acids obtained by the hydrolysis of the proteins in the extract e.g. hydrolysed collagen.

4. The applicant mentions that foods containing SH are very limited and only rooster combs and viscera have high amounts of this substance. These sources of SH are not consumed in all European countries and the applicant therefore proposes to incorporate RCE into different foods which are consumed daily in Europe as a way of providing additional sources of SH in order to support joint health in the general population.

5. RCE has been classified as a complex novel food from non-GM source, the source of the novel food has a history of food use in the EU (class 2.1) according to the scheme in Commission Recommendation 97/618 (EC).

I. Specification of the novel food

Information on this aspect is provided on p. 11-21 of the application dossier

6. The chemical and physical specification for RCE has been established by the applicant and can be found in the table below.

SPECIFICATIONS	LIMITS	METHODS
Glucuronic acid content (expressed as sodium hyaluronate)	60 - 80 %	Eur. Ph. Monograph 1472
Appearance	White or almost white hygroscopic powder	Visual
pH	5.0 – 8.5	Eur. Ph. 2.2.3
Chlorides	Not more than 1 %	Mohr Method
Nitrogen	Not more than 8 %	Eur. Ph. 2.5.9
Loss on drying	Not more than 10 %	Eur. Ph. 2.2.32
Heavy metals	Not more than 10 ppm	USP <231>
Mercury	Not more than 0.10 ppm	Eur. Ph. 2.2.58
Arsenic	Not more than 1 ppm	Eur. Ph. 2.2.58
Cadmium	Not more than 1 ppm	Eur. Ph. 2.2.58
Chromium	Not more than 10 ppm	Eur. Ph. 2.2.58
Lead	Not more than 0.5 ppm	Eur. Ph. 2.2.58
Dioxins and furans	Not more than 2.0 pg/g	EPA* Method 1613
PCB's	Not more than 4.0 pg/g	EPA* Method 1613
MICROBIOLOGICAL PARAMETERS		
Total viable aerobic count	Not more than 10^2 cfu/g	Eur. Ph. 2.6.12
Escherichia coli	Absence/ g	Eur. Ph. 2.6.13
Salmonella sp.	Absence/ g	Eur. Ph. 2.6.13
Staphylococcus aureus	Absence/ g	Eur. Ph. 2.6.13
Pseudomonas aeruginosa	Absence/ g	Eur. Ph. 2.6.13

7. The applicant has provided data from analyses carried out on ten independent lots of RCE (Annex 1, p16-17) which demonstrate that all lots conformed with the specifications. Some parameters e.g. specific heavy metals (mercury, arsenic, cadmium, chromium and lead), dioxins, furans and PCBs were not analysed for every single batch, as the applicant states that the safety and quality of RCE is well established and the analysis of these parameters is done only twice a year to assure that these substances are absent. However, no less than three batches were analysed for each specification parameter.

***Discussion**: The Committee did not raise any concerns relating to this section of the dossier.*

II. Effect of the production process applied to the novel food

Information on this aspect is provided on p 22-34 of the application dossier

8. RCE is produced by an extraction process from rooster combs, using enzymatic hydrolysis and subsequent concentration and precipitation of the product.

9. The production process is detailed in the dossier (Annex 1, p22-25, protected information).

10. Studies under accelerated storage conditions (40 ± 2ºC / 75 ± 5% Relative Humidity, RH, for 6 months) and long-term storage conditions (25 ± 2ºC / 60 ± 5% RH, 40-43 months) have been conducted with three different production batches of RCE. The applicant states that storage under these conditions, using as a primary packaging a triple LDPE bag, and a metal drum as a secondary packaging, did not compromise the stability of the RCE.

11. The stability of different concentrations of RCE in yoghurts was assessed under refrigerated storage conditions for 1 and 1.5 months, which covers the mean shelf life of a standard commercial yogurt (normally three weeks). Analyses show that RCE remained stable with only minor variations in concentration, which according to the applicant are considered acceptable, compared to the initial theoretical concentration. Moreover, the presence of the RCE did not cause any microbiological presence after 1.5 months.

***Discussion**: The Committee did not raise any safety concerns regarding the production process. The issue of animal welfare during the production of RCE was raised during the public consultation and also by the Committee. The applicant has clarified that rooster combs are obtained from authorized slaughterhouses that slaughter poultry for human consumption. Combs are obtained post-mortem from poultry that undergo ante and post-mortem veterinary controls and are declared as fit for human consumption, the applicant has provided a certificate from the slaughterhouse where the combs are obtained. The Committee was satisfied that there are no outstanding concerns relating to animal welfare.*

III. History of the organism used as a source of the novel food

Information on this aspect is provided on p 35-36 of the application dossier

12. RCE is obtained from an edible non-GM biological source (rooster combs from *Gallus gallus*). The source organism is fully characterized and this and/or the food obtained from it are not detrimental to human health according to the applicant. Rooster combs have a long established human consumption in Europe and continue to be part of the normal diet in some countries, including frequently consumed dishes such as home-made recipes (stews) and

industrially prepared soup concentrates. They are considered a delicacy in restaurants in countries such as France and Spain. The applicant states that first evidence of the use of rooster combs is found in medieval recipe books from the 15th century. *Gallus gallus* combs used as the source of the novel ingredient are declared as fit for human consumption.

13. Rooster comb is a moderately thin, fleshy formation of smooth soft surface texture, firmly attached from the beak along the top of the skull with a strong base. Rooster comb can measure more than 7 cm in length and weigh more than 8 grams.

Discussion: The Committee did not raise any concerns relating to this section of the dossier.

IX. Anticipated intake/extent of use of the novel food

Information on this aspect is provided on p 37-42 of the application dossier

14. RCE is proposed for use in milk-based fermented beverages, yogurts, milks and fromage frais for the general population, with the exception of pregnant women, children and people allergic to sodium hyaluronate and/or avian proteins. These products are intended to be taken in one daily serving containing 80 mg of RCE.

15. The applicant intends that RCE-containing products will be consumed by the adult population, sportsmen, the elderly, and menopausal women. The Secretariat has asked the advice of the Medicines and Healthcare products Regulatory Agency, who advised that sodium hyaluronate from RCE, or from any other source, would not be regarded as medicinal. The applicant is aware that any claims relating to maintaining joint health may be regarded as health claims and require approval under the EU Nutrition and Health Claims Regulation (Regulation (EC) No 1924/2006).

16. RCE's components are present in a comb at an approximate proportion of 1%. The applicant states that 25 g of rooster combs (considering a meal portion of 3 combs of approximately 8 g per comb) contain 250 mg of the components found in the extract. The recommended daily dose (80 mg) is therefore equivalent to consumption of a single comb.

17. In order to calculate the maximum estimated consumption of the RCE, it has been assumed that all dairy products consumed daily would contain the extract. Predicted total dairy intake for European countries has been obtained from the FAOSTAT (Food and Agriculture Organization of the United Nations) database.

18. In countries with the highest total dairy intake, namely Finland (975.34 g/capita day) or Sweden (1032.88 g/capita/day), the inclusion of RCE in all dairy products would result in an intake of 0.624 g/capita/day of RCE for Finland and 0.661 g/capita/day for Sweden.

***Discussion**: Members requested that the applicant provides a more complete set of intakes data taking into account non-target groups such as children. The applicant stated that it intends to label foods containing RCE to reduce the likelihood of consumption by non-target groups such as children and pregnant women. The applicant acknowledged that it is nevertheless possible that children may consume RCE-containing foods e.g. fromage frais on occasions. The applicant therefore calculated an estimated daily intake of RCE on the basis of mean consumption of dairy products by schoolchildren (aged 4-10) and toddlers (aged 12m). Even in the worst case scenario estimation (i.e. assuming that all dairy desserts would contain RCE, which is not a likely scenario), the estimated daily intake of RCE would be less than 2.4 mg/kg bodyweight/day for children and 3.8 mg/kg bw/day for toddlers. The Committee also considered estimates based on high level consumption of yoghurt and fromage frais by toddlers, provided by the Food Standards Agency using data from the British National Diet and Nutrition Survey. This analysis showed that the intake of RCE could be up to 9.3 mg/kg bodyweight/day.*

X. Information from previous human exposure to the novel food or its source

Information on this aspect is provided on p 43-46 of the application dossier

19. The applicant notes that rooster combs have been widely consumed in the EU. Also, there are several food supplements on the EU market (Belgium, France, Germany, Ireland, Italy, Portugal, Spain, and UK), containing sodium hyaluronate. According to the applicant, these supplements do not specify the source of sodium hyaluronate except one which is obtained by microbial fermentation, and no adverse effects have been reported.

***Discussion:** The Committee did not raise any concerns about this section of the dossier.*

XI. Nutritional information on the novel food

Information on this aspect is provided on p 47-49 of the application dossier

20. The applicant states that RCE in dairy products is not intended to replace any existing food ingredient. The applicant provided nutritional information for skimmed yogurt, for RCE and for RCE-supplemented skimmed yogurt. The quantity of RCE added to the yogurt is very low (80 mg per portion) and will not have any nutritional impact on a balanced diet. The only nutritional parameter of the yoghurt which is increased by adding RCE is sodium (3% increase

relative to non-supplemented yogurt), but the supplemented yogurt remains a "low sodium" food (72.25 mg per 125 g of yogurt).

Discussion: *The Committee did not raise any concerns about this section of the dossier.*

XII. Microbiological information on the novel food

Information on this aspect is provided on p. 50 of the application dossier

21. The applicant has provided microbiological specifications and has also supplied results of analyses for ten independent lots of RCE. All batches comply with the specifications.

22. The applicant states that RCE is manufactured using Good Manufacturing Practice and is obtained from animals declared fit for human consumption. The applicant has also provided a viral safety report. Stability studies conducted on RCE-supplemented yoghurt indicate that addition of RCE to yoghurt does not promote the presence of pathogenic organisms.

Discussion*: The applicant confirmed to the Committee that all tests for potential pathogenic micro-organisms indicated that the relevant species were absent and the Committee was satisfied that the microbial composition of yoghurt was not significantly changed by the addition of the novel ingredient.*

XIII. Toxicological information on the novel food

Information on this aspect is provided on p. 57-87 of the application dossier

23. The applicant has conducted a range of toxicity studies which are summarised below. The applicant concludes that these studies demonstrate that the extract is safe and rule out any toxicological concerns relating to RCE. The No Observed Adverse Effect Level (NOAEL) established from these toxicity studies is 600 mg/kg/day, which is the highest dose used in the feeding studies. For a 60 kg adult, this would equate to approx. 5.76 g/capita/day of RCE, according to the dose extrapolation method of Reagan Shaw *et al*., 2007.

24. In their application dossier (section IX.3) the applicant estimated the "worst-case" intake of RCE in different EU member states, based on the extreme assumption that RCE is added to all dairy products, and showed that the resulting intakes would be between 4.5% (for Bulgarian consumers, 0.263 g RCE/day) and 11.4% (for Swedish consumers, 0.661 g RCE/day) of the human equivalent of the NOAEL.

Study Title	**Type**	**Subject studied**	**Route of Admin-istation**	**Dose**	**Safety conclusions drawn by applicant**
Genotoxicity study	In vitro	Salmonella, E.coli	-	5 concen-trations	No toxicity in any of the strains, no mutagenic responses
Acute oral toxicity study in rats	In vivo	18 rats	Oral (gastric gavage)	1000mg/kg, 2000mg/kg	No mortality at 2000 mg/kg, No clinical signs during or after treatment.
2 week dose range finding study	In vivo	40 rats	Oral (gastric gavage)	200, 400, 600 mg/kg/day	No mortality neither alterations in feed consumption, body weight or necropsies, no clinical signs observed
Oral toxicity by 4 weeks repetitive administration	In vivo	100 rats	Oral (gastric gavage)	5, 55, 600 mg/kg/day	No mortality neither alterations in feed consumptions, body weight or necropsies. No clinical or histological signs observed.
13-week oral (gavage) toxicity in rats with a 4-week recovery period	In vivo	100 rats	Oral (gastric gavage)	5, 55, 600 mg/kg/day	No mortality neither alterations in feed consumption, body weight or necropsies No clinical or histological signs observed.
Acute intraperitoneal toxicity in rat	In vivo	26 rats	Intra-peritoneal	250, 500, 900, 1000 mg/Kg/day	No mortality observed. Observed clinical signs post administration as abnormal locomotion, piloerection. Minimum Lethal Dose of the RCE established is more than 1000 mg/Kg
Study of the intestinal absorption of RCE	In vitro	6 rats	-	Solution of 200 µg/ml	The RCE is absorbed from the media through the intestinal mucous. The most important absorption occurs in the duodenum
Study of the effects of the RCE on Hyaluronic Acid concentration in a horse model. (60 days administration)	In vivo	12 horses	Oral	250 mg/day	No adverse events related to the study products were observed. No significant changes were observed in plasma and synovial fluid analyses. Treated horses presented higher levels of hyaluronate in the synovial fluid.
Clinical trial on efficacy and safety of RCE (8 weeks administration)	In vivo	20 adults	Oral	80 mg/day	No serious adverse events were reported. The RCE appeared to be well tolerated and safe. No alterations in body weight, vital signs, and safety laboratory results.

Study Title	Type	Subject studied	Route of Admin-istation	Dose	Safety conclusions drawn by applicant
Clinical trial evaluating the efficacy and safety of a yoghurt supp-lemented with RCE.	In vivo	40 adults	Oral	80 mg/day	No significant changes in body weight or clinical parameters as pulse rate or blood pressure were observed.

***Discussion**: Members questioned the use of the Reagan Shaw et al. method by the applicant and viewed the use of this method as rather unusual in the context of food-related exposure assessments. Members requested an explanation for using this method rather than conventional safety factors. The applicant explained that the method described by Reagan Shaw et al. provides a means of converting the dose of a substance used in animal studies into the Human Equivalent Dose (HED) using inter-species factors based on body surface area. This body surface area approach is recommended in US FDA guidance for industry when estimating the safe starting dose for clinical trials (after the incorporation of a suitable safety factor).*

The NOAEL for RCE, based on animal feeding studies, is 600 mg/kg bodyweight/day. The applicant calculated that the human equivalent dose is 5.76 g/capita/day for an adult weighing 60 kg, (i.e. 96 mg/kg bodyweight/day). This calculation does not include a safety factor.

Although the applicant did not specifically argue against the conventional "ADI" approach, which is generally used for substances in food, they argue that a 100-fold safety factor would be excessive in light of the properties of hyaluronic acid, the main component of RCE.

Using a conventional food safety approach, and without making the adjustment for body surface area, the Food Standards Agency calculated that the applicant's "worst case" intake assessments provide a safety factor of between 54 (for Swedish consumers, 0.661 g RCE/day) and 137 (for Bulgarian consumers, 0.263 g RCE/day) when compared with the NOAEL from the animal feeding studies, assuming an adult body weight of 60kg.

Members were satisfied that there were no outstanding questions relating to this section of the dossier. While it was possible that the safety margin between intake of RCE by toddlers and the NOAEL from animal feeding studies would be less than 100, this intake represented a worst case scenario involving a combination of assumptions that was extremely unlikely to occur in practice. The Committee therefore concluded that there was no significant concern relating to consumption by children, but advised that any future request for a wider range of uses of this ingredient should be accompanied by a better assessment of intake.

The Committee emphasised that its assessment of RCE focussed solely on safety and does not endorse any health benefits mentioned by the applicant. Efficacy assessment does not fall within the remit of the novel foods regulation and is therefore not part of the Committee's role.

XIV. Allergenicity and labelling

Information on this aspect is provided on p.38 and p. 44 of the application dossier

25. The applicant stated in the dossier that no allergic episodes have been described in the human and animal studies as a result of RCE supplementation. RCE contains sodium hyaluronate (60-80%), glycosaminoglycans (about 20%) and partially hydrolyzed proteins (about 20%). Both sodium hyaluronate and glycosaminoglycans according to the applicant have a broad history of use in the EU market (as oral food supplements) without any documented adverse reports related to allergenicity. The proteins present in the RCE are partially hydrolyzed, with a mean molecular weight of 1234 ± 5 Da, and for this reason the applicant states that their allergenic potential is very low.

26. The applicant acknowledges that in theory there could be some cases of hypersensitivity to sodium hyaluronate or avian proteins. Thus, the applicant proposed to include a warning label for RCE-containing foods for people allergic to sodium hyaluronate and/or avian proteins to illustrate that RCE-containing foods are unsuitable for such individuals.

***Discussion**: The Committee stated that, in the absence of evidence that components of RCE posed a risk, the applicant's proposal to label foods containing RCE as unsuitable for those with allergies to avian proteins was too restrictive and will limit consumer choice, perhaps unnecessarily. The applicant therefore agreed to determine experimentally whether the hydrolysed proteins in RCE have the ability to cross-react with egg proteins that are known to elicit allergic reactions. This was done using indirect inhibition ELISA to investigate the ability of RCE to bind serum IgE from egg allergic patients.*

The applicant reported that none of the three batches of RCE tested showed any capacity to bind to IgE from pooled sera of patients with egg allergy. The applicant also highlighted the relatively small size of the hydrolysed proteins in RCE and the fact that RCE is derived from connective tissue (mainly collagen) which is known to be less allergenic than egg. The Committee concluded that these additional data were of high quality and provided adequate reassurance that the proteins in RCE were unable to cross-react with egg proteins.

Although not a safety-related issue, Members were interested in more detail about the source of the sera used in the ELISA and whether these samples were obtained with ethical consent. The applicant confirmed that the sera were sourced in an ethical way and provided documentation to support this. The study centre CIAL (the Institute of Food Science Research of the Spanish National Research Council) was also granted authorisation from the corresponding bioethics committee. The Committee was satisfied with the applicant's responses.

Although no further information was requested from the applicant relating to labelling, the Committee highlighted the need for suitable labelling of RCE-containing foods to alert non-target groups and vegetarians to the presence of the novel ingredient. As it is a product of animal origin, the source of RCE needs to be clearly stated if it is used in foods that are otherwise regarded as suitable for vegetarians, such as dairy products.

CONCLUSION

The ACNFP has completed its assessment of RCE as a novel ingredient to be added to a range of foods and did not have any significant safety concerns relating to this ingredient.

During its assessment of RCE, the Committee requested further information from the applicant on the following:

- Allergenicity
- Toxicology
- Intakes
- Microbiological information
- Animal welfare issues

After reviewing the applicant's responses to these issues, the Committee did not have any outstanding safety concerns.

The Committee has also reviewed public comments relating to the dossier that were received during a public consultation and has considered these as part of its assessment.

The Committee emphasised that this assessment was based purely on safety and does not extend to assessing or endorsing any health benefits that have been mentioned by the applicant.

The Committee therefore concluded that RCE, added to milk-based fermented beverages, yogurts, milks and fromage frais at the levels proposed by the

applicant, is unlikely to present a health risk to consumers. The Committee emphasised that if the novel ingredient is authorised in the EU, foods into which it is incorporated should be clearly labelled so as not to mislead consumers. Particular care should be taken to inform consumers of the source of the ingredient if it is added to products that are otherwise regarded as suitable for vegetarians.