Bureau Nieuwe Voedingsmiddelen
Novel Foods Unit

Hanenkamextract

Rooster combs extract

Tweede beoordeling van de veiligheid voor de consument, volgens de Europese verordening 258/97 betreffende nieuwe voedingsmiddelen en nieuwe voedselingrediënten

Second opinion regarding consumer safety, in accordance with European Regulation 258/97 concerning novel foods and novel food ingredients

aan/to:

de Minister van Volksgezondheid, Welzijn en Sport
the Minister of Health, Welfare and Sport

Nr. 2012-01 BNV, Den Haag, 6 januari 2012
No. 2012-01 BNV, The Hague, January 6, 2012

Bureau Nieuwe Voedingsmiddelen
CBG
Postbus 16229
2500 BE Den Haag
novelfoods@cbg-meb.nl
www.nieuwevoedingsmiddelen.nl

Novel Foods Unit
MEB
P.O. box 16229
2500 BE The Hague
The Netherlands
novelfoods@cbg-meb.nl
www.novel-foods.nl

Inhoudsopgave

Contents

Beoordeling

Inleiding

Aan de orde is een tweede beoordeling volgens de Europese Verordening 258/97 (EG97, EG97a), over het gebruik als nieuw voedingsmiddel van een extract uit hanenkammen. Het bevat voornamelijk natriumhyaluronaat, een bestanddeel van de bindweefselmatrix. De aanvraag is ingediend door Bioiberica SA uit Spanje en betreft het gebruik van dit extract als nieuw ingrediënt in allerlei zuivelproducten.

In het kader van de desbetreffende Europese toelatingsprocedure is deze tweede beoordeling uitgevoerd door het Bureau Nieuwe Voedingsmiddelen van het College ter Beoordeling van Geneesmiddelen. Het bureau heeft hiervoor de Commissie Veiligheidsbeoordeling Nieuwe Voedingsmiddelen geraadpleegd, hierna genoemd 'de commissie VNV'.

Eerste beoordeling

De eerste beoordeling van de aanvraag voor markttoelating is verricht in het Verenigd Koninkrijk door de *Advisory Committee on Novel Foods and Processes* (ACNFP). In het rapport van de eerste beoordeling concludeert de ACNFP dat hanenkamextract veilig kan worden geconsumeerd bij de toepassing die de aanvrager voorstelt. De ACNFP benadrukt dat het nieuwe ingrediënt, als het wordt toegelaten, duidelijk moet worden geëtiketteerd om consumenten te informeren over de bron van het extract. Zij noemt in het bijzonder producten die zonder toevoeging geschikt zijn voor vegetariërs. Ook is het belangrijk om zo consumenten te waarschuwen die mogelijk allergisch zijn voor kippenvlees. De ACNFP wijst erop dat zij de gezondheidsvoordelen die de aanvrager vermeldt niet heeft geëvalueerd.

Bevindingen van de Commissie VNV

De Commissie VNV maakt bezwaar tegen de toelating als nieuw voedingsmiddel van het extract van hanenkammen, omdat er onvoldoende gegevens zijn om het product te kunnen beoordelen. De commissie VNV heeft haar oordeel gebaseerd op de informatie in het dossier, waarvan de samenvatting is opgenomen als bijlage A, aanvullende informatie van de firma aan de ACNFP (Bio11), en de eerste beoordeling door de ACNFP, toegevoegd als bijlage B.

Productspecificatie. Volgens de beschrijving in het dossier en de eerste beoordeling bevat het product 60-80 % natriumhyaluronaat. De aanvrager karakteriseert het molecuul hyaluronaat als een onvertakte keten van disacchariden die aan elkaar zijn gekoppeld door ß(1-4)glycosidische bindingen. Het repeterende disaccharide bestaat uit natrium-D-glucuronaat dat via een ß(1-3)glycosidische binding is gekoppeld aan een N-acetyl-D-glucosamine. Daarnaast is er ongeveer 20 % glycosamineglycanen aanwezig. Volgens de aanvrager bestaat deze fractie uit essentiële structurele componenten van kraakbeen zoals chondroïtinesulfaat en dermatansulfaat. Ook bevat het extract ongeveer 20 % eiwit dat gedeeltelijk is gehydrolyseerd tot kortere peptiden en aminozuren, bijvoorbeeld afkomstig van collageen. Dit gehydrolyseerd eiwit heeft volgens de aanvrager een gemiddeld molecuulgewicht van ongeveer 1234 Da.

De specificatie van het product vermeldt het gehalte natriumhyaluronaat zoals dat is afgeleid van het gemeten glucuronzuurgehalte. Volgens de commissie VNV wordt met een

dergelijke analysemethode echter het totaal gehalte aan glycosamineglycanen gemeten inclusief gesulfateerde glycosamineglycanen[1] zoals bijvoorbeeld het chondroïtinesulfaat dat de aanvrager vermeldt. De commissie VNV merkt op dat eiwitten niet apart zijn opgenomen in de specificatie, wel het stikstofgehalte (≤ 8 %). Het vochtgehalte is maximaal 10%. Daarnaast zijn in de specificatie van het product grenswaarden opgenomen voor enkele zware metalen, voor dioxinen en furanen, voor PCBs en voor aantallen van verschillende soorten micro-organismen.

De commissie VNV wijst op een andere weergave van de samenstelling in Tabel 8 van het dossier. Hier is niet duidelijk hoe het vezelgehalte (36 g/100 g) is gedefinieerd en lijkt het stikstofgehalte (44 g/100 g) vele malen hoger dan gespecificeerd in de Tabellen 1-3. Volgens de commissie zijn de gegevens over het stikstofgehalte niet eenduidig omdat de analysen die de aanvrager heeft uitgevoerd geen onderscheid maken tussen stikstofmoleculen afkomstig van eiwit of de N-acetyl-glucosamine eenheid van hyaluronaat (en andere glycosamineglycanen). Hiermee samenhangend ontbreken betrouwbare waarden voor het maximum eiwitgehalte van het extract. Met het oog op mogelijke allergeniteit is de grootteverdeling van de hydrolysefragmenten van het eiwit ook van belang. Samenvattend concludeert de commissie VNV dat de samenstelling van het product onvoldoende duidelijk is.

Productieproces. Het dossier vermeldt dat de hanenkammen die voor de productie worden geëxtraheerd, afkomstig zijn van hanen (*Gallus gallus*) uit bevoegde slachthuizen. Het productieproces begint met een enzymatische hydrolyse waarna er een aantal bewerkingen worden gedaan om het product te concentreren en te drogen.

De ACNFP heeft over het productieproces en ook over de stabiliteit van het extract geen opmerkingen ten aanzien van de veiligheid.

De commissie VNV vindt het productieproces dusdanig beknopt weergegeven dat zij niet kan beoordelen of het totaal aan bestanddelen van het extract overeenkomt met ongeveer 1% van de hanenkam zoals de aanvrager beweert. De aanvrager verklaart dat het gebruikte enzym van levensmiddelenkwaliteit is en verwijst hierbij naar certificaten van de producent, maar volgens de commissie VNV onderbouwen deze documenten dit niet. De aanvrager vermeldt dat er tijdens het proces een hittebehandeling plaatsvindt die het gebruikte enzym moet inactiveren. Echter, deze omstandigheden zijn niet gespecificeerd en bovendien onderbouwen de summiere gegevens op pagina 23 in het dossier onvoldoende de effectiviteit van deze inactivatiestap. Verder constateert de commissie VNV dat op basis van de beschikbare informatie niet duidelijk is of het geïnactiveerde enzym wordt verwijderd. Het is daardoor onbekend in hoeverre dit enzym bijdraagt aan het totale eiwitgehalte. Bovendien is het van belang dat de aanvrager de hittebehandeling zorgvuldig controleert om te garanderen dat het krachtige enzym volledig is geïnactiveerd.

Geschatte inname. De aanvrager wil het hanenkamextract gaan verwerken in allerlei levensmiddelen, als eerste in melk, kwark, yoghurt en gefermenteerde melkdranken bedoeld voor de algemene bevolking met uitzondering van zwangere vrouwen en kinderen. Als

[1] http://en.wikipedia.org/wiki/Glycosaminoglycan

doelgroep noemt de aanvrager volwassenen, sporters, ouderen en vrouwen in de overgang. Men is van plan 80 mg extract te verwerken in één dagelijkse portie zuivel waarvoor in het dossier 125 g yoghurt of 50–100 g kwark als voorbeeld wordt gebruikt (Bijlage A, Bio11). Voor andere type producten worden geen extractgehalten vermeld. Gebaseerd op die EU landen waar de meeste zuivel wordt geconsumeerd, heeft de aanvrager berekend dat de hoogste dagelijkse inname van het hanenkamextract uitkomt op ongeveer 640 mg per hoofd van de bevolking. Hierbij is men uitgegaan van een totale dagelijkse zuivelinname van ongeveer 1000 g zuivel per inwoner van Finland en Zweden, afkomstig van voedselbeschikbaarheidgegevens (FAOSTAT database) en aangenomen dat elk geconsumeerd product het nieuwe extract bevat. Daarnaast heeft de aanvrager op basis van Engelse voedselconsumptiegegevens uit 2008 -2009 berekend, dat jonge kinderen (1-10 jaar) die onbedoeld toch zuiveltoetjes eten met het hanenkamextract, gemiddeld 34-38 mg extract per dag kunnen binnenkrijgen (Bio11). Volgens de ACNFP is er echter bij hele jonge kinderen die liefhebbers zijn van yoghurt en kwarkproducten een veel hogere blootstelling mogelijk die neerkomt op ruim 9 mg extract per kg lichaamsgewicht per dag.

In aanvulling op de gegevens in het dossier en eerste beoordeling, verwijst de commissie VNV naar recente Nederlandse voedselconsumptiegegevens voor verschillende typen zuivelproducten (VCP11). Uit het onderzoek bij 7-69 jarige Nederlanders blijkt dat de 95 percentiel van de totale zuivelinname bij verschillende leeftijdsgroepen varieert van 721 tot 1040 g per dag. In het algemeen draagt consumptie van yoghurt voor minstens de helft bij aan de totale zuivelinname, ook bij hele jonge kinderen (VCP08). Over deze laatste groep is de commissie VNV het eens met de ACNFP dat zij veel meer van het extract kunnen binnen-krijgen dan waar de aanvrager vanuit gaat (Bio11). De commissie VNV constateert echter dat zonder een duidelijke specificatie van de extractgehalten in producten die de aanvrager voorstelt, een betrouwbare innameschatting van het nieuwe product niet mogelijk is.

Eerder gebruik. De aanvrager noemt dat hanenkammen in de EU worden geconsumeerd veelal als onderdeel van traditionele gerechten. Ook is er op de Europese markt een verscheidenheid aan voedingssupplement die natriumhyaluronaat bevatten, maar de aanvrager heeft geen informatie over de bron hiervan op één product van microbiologische oorsprong na.

Net als de ACNFP heeft de commissie VNV hierbij geen opmerkingen.

Microbiologische informatie. De aanvrager heeft in de productspecificatie een aantal microbiologische parameters opgenomen en verstrekt daarbij ook de negatieve testresultaten van meerdere productiepartijen. De hanenkammen zijn afkomstig van dieren waarvoor een verklaring is afgegeven dat deze geschikt zijn voor menselijke consumptie. Het dossier bevat tevens de resultaten van onderzoek met modelvirussen om te illustreren dat potentieel aanwezige virussen effectief kunnen worden geïnactiveerd tijdens het productieproces.

Net als de ACNFP meent de commissie VNV dat de aanvrager voldoende heeft aangetoond dat relevante soorten pathogene micro-organismen afwezig zijn. Ook is men gerustgesteld dat de microbiële samenstelling van de yoghurt niet wordt beïnvloed door toevoeging van het extract.

Toxicologische informatie. In het dossier en de eerste beoordeling wordt de vertering van het hanenkamextract niet besproken. Wel refereert de aanvrager naar een *in vitro* onderzoek van de absorptie van het hanenkanextract door de darmmucosa (Tabel 14), maar hiervan zijn geen resultaten in het dossier opgenomen. Er zijn de commissie VNV geen gegevens bekend over hoe hyaluronaat in het maag-darmkanaal wordt afgebroken.

De aanvrager heeft het hanenkamextract onderzocht op mogelijke toxische effecten in een genotoxiciteitstest met bacteriën en in een aantal proefdieronderzoeken. Daarnaast zijn ook enkele onderzoeken naar vermeende gunstige effecten in paarden en mensen beschreven. Dit is samengevat in Tabel 14 van het dossier en overgenomen in het rapport van de eerste beoordeling. De ACNFP is het eens met de aanvrager dat er geen aanwijzingen voor schadelijke effecten zijn en beschouwt de hoogst geteste dosis in het 90-dagen onderzoek met ratten als de NOAEL, te weten 600 mg/kg BW/dag. Op basis hiervan heeft de ACNFP berekend dat de veiligheidsmarge varieert van 54 tot 137 gebaseerd op respectievelijk de hoogst en laagst geschatte inname volgens het worstcasescenario van de de aanvrager. De ACNFP meent dat het uiterst onwaarschijnlijk is dat voor peuters de veiligheidsmarge kleiner zal zijn dan 100 en is daarom niet noemenswaardig bezorgd over de veiligheid van kinderen die het hanenkamextract onbedoeld binnenkrijgen.

De commissie VNV is het eens met de ACNFP dat uit de beschikbare resultaten van de toxicologische onderzoeken geen punten van zorg naar voren komen. Wel is het de commissie VNV opgevallen dat de aanvrager van het 90-dagen onderzoek met ratten alleen een conceptrapport heeft verstrekt. Het uitgevoerde onderzoek lijkt gezien de pathologie compleet, maar het is niet duidelijk waarom de OECD richtlijn 452 voor chronisch onderzoek hier is gebruikt. Gezien de beperkte veiligheidsgegevens is een ondertekende eindversie van dit essentiele onderzoek noodzakelijk voor een definitief oordeel.

Allergeniteit. De aanvrager had oorspronkelijk voorgesteld een waarschuwing te plaatsen op het etiket van levensmiddelen met het extract, dat dit product niet geschikt is voor mensen die allergisch zijn voor natriumhyaluronaat of vogeleiwitten. De ACNFP meent dat dit consumenten mogelijk onnodig beperkt in hun keuze omdat het erg onwaarschijnlijk is dat het nieuwe product een allergische reactie uitlokt bij personen die overgevoelig zijn voor ei.

De commissie VNV is het niet geheel eens met de ACNFP dat een waarschuwing "niet geschikt voor personen met een ei-allergie" overbodig zou zijn. De commissie vindt dat de aanvullende gegevens van de aanvrager (Bio11) onvoldoende zekerheid bieden over de afwezigheid van serologische kruisreactiviteit van IgE anti-kippenei-eiwit met eiwitten in het extract, omdat de grootte en kenmerken van de gebruikte serumpool van patiënten met een allergie voor kippenei-eiwit niet gedefinieerd is. Voor consumenten die allergisch zijn voor kippenvlees (zeldzaam in vergelijking met ei-allergie) vindt de commissie VNV net als de ACNFP dat een waarschuwing gerechtvaardigd is. Omdat het hanenkamextract niet is onderzocht met sera van dergelijke personen is kruisreactiviteit niet uit te sluiten is.

Conclusie

Volgens de ACNFP is het onwaarschijnlijk dat het gebruik van hanenkamextract in zuivelproducten zoals voorgesteld in het dossier een gezondheidsrisico met zich mee brengt voor de consument. Hoewel de commissie VNV geen concrete aanleiding tot bezorgdheid heeft over de veiligheid van dit nieuwe product, is zij het niet eens met het positieve eindadvies van de ACNFP omdat er bij een aantal onderdelen in het dossier verschillende

niet onbelangrijke tekortkomingen zijn geconstateerd. De commissie VNV is daarom van mening dat aanvullende informatie van voldoende kwaliteit noodzakelijk is voordat zij kan instemmen met een toelating.

Referenties

Bio11 Answers to the ACNFP comments on the application for the approval of a Rooster/Cockerel Combs Extract (RCE) as a novel food ingredient. Bioiberica, March 5, 2011.

EG97 Verordening (EG) nr. 258/97 van het Europees Parlement en de Raad van 27 januari 1997 betreffende nieuwe voedingsmiddelen en nieuwe voedselingrediënten. Publicatieblad van de Europese Gemeenschappen 1997; L43: 1-6.

EG97a Aanbeveling (EG) nr. 97/618/EG van de Commissie van 29 juli 1997 betreffende de wetenschappelijke aspecten en de presentatie van de informatie die nodig is om aanvragen voor het in de handel brengen van nieuwe voedingsmiddelen en nieuwe voedselingrediënten te ondersteunen alsmede het opstellen van de verslagen van de eerste beoordeling uit hoofde van Verordening (EG) nr. 258/97 van het Europees Parlement en de Raad. Publicatieblad van de Europese Gemeenschappen 1997; L253: 1-36.

VCP08 VCP-Jonge kinderen (2005-2006).
Dutch National Food Consumption Survey -Young Children 2005/2006, see http://www.rivm.nl/bibliotheek/rapporten/350070001.pdf

VCP11 VCP-Basisgegevensverzameling (2007-2010).
Dutch National Food Consumption Survey 2007-2010. Diet of children and adults aged 7 to 69 years, see http://www.rivm.nl/bibliotheek/rapporten/350050006.pdf

Voor overzicht voedselconsumptiepeilingen in Nederland, zie
http://www.rivm.nl/Onderwerpen/Onderwerpen/V/Voedselconsumptiepeiling/Overzicht_peilingen

Assessment

Introduction

This report describes a second assessment under European Regulation 258/97 (EG97, EG97a) of the use as a novel food of an extract from rooster combs. The extract consists mainly of sodium hyaluronate, a component of the matrix of connective tissues. The application was made by the Spanish company Bioiberica SA, and related to the use of the extract as novel ingredient in a wide range of dairy products.

The second assessment reported here was performed by the Novel Foods Unit of the Medicines Evaluation Board, in accordance with the European authorisation procedure. The Unit consulted the Committee on the Safety Assessment of Novel Foods (referred to below as 'the VNV Committee') regarding its assessment.

Initial assessment

The initial assessment of the application for market authorisation was conducted in the United Kingdom by the Advisory Committee on Novel Foods and Processes (ACNFP). In the report on its initial assessment, the ACNFP concludes that rooster combs extract can safely be consumed in the manner described in the dossier. The ACNFP nevertheless stresses that, if the novel ingredient is approved, the applicant will have to ensure that all products made with it are clearly labelled to inform consumers about the source of the extract. In that context, particular reference was made to products that, without addition of the extract, would be suitable for vegetarians. The importance of warning consumers who might be allergic to chicken meat was also highlighted. The ACNFP pointed out that it had not evaluated the health benefits claimed by the applicant.

Findings of the VNV Committee

The VNV Committee objects to rooster combs extract being authorised for use as a novel food, because insufficient data are available to permit the assessment of the product. The VNV Committee based its assessment on the information in the dossier, which is summarised in Annex A, additional data submitted to the ACNFP by the applicant (Bio11), and the initial assessment by the ACNFP, which forms Annex B.

Product specification. According to the information in the dossier and the initial assessment, the product contains 60-80% sodium hyaluronate. The applicant describes the hyaluronate molecule as an unbranched chain of disaccharides linked together by ß(1-4)glycosidic bonds. The repeating disaccharide consists of sodium D-glucuronate, which is linked to an N-acetyl-D-glucosamine by a ß(1-3)glycosidic bond. In addition, the product contains about 20% glycosaminoglycans. According to the applicant, this fraction consists of essential structural cartilage components, such as chondroitin sulphate and dermatan sulphate. Roughly a further 20% of the product is accounted for by protein, which is partially hydrolysed to shorter peptides and amino acids originating from sources such as collagen. This hydrolysed protein is said by the applicant to have an average molecular weight of roughly 1234 Da.

The sodium hyaluronate concentration cited in the product specification was deduced from the measured glucuronic acid concentration. However, the VNV Committee makes the point that the analysis method used can indicate only the overall glycosaminoglycan

concentration, including sulphated glycosaminoglycans[1] such as the chondroitin sulphate referred to by the applicant. The VNV Committee also notes that proteins are not specified separately, although the nitrogen content (≤ 8%) is. The moisture content of the product is no more than 10%. The applicant has also specified limits for various heavy metals, dioxins and furans, PCBs and maximum counts for various species of micro-organisms.

The VNV Committee additionally highlights another statement of the product's composition in Table 8 of the dossier. From this statement, it is not clear how the fibre content (36 g/100 g) has been determined, and the cited nitrogen content (44 g/100 g) is many times higher than that specified in Tables 1–3. The Committee regards the data on the nitrogen content as ambiguous, because the applicant's analyses do not distinguish between nitrogen molecules originating from protein and the N-acetyl-glucosamine component of hyaluronate (and other glycosaminoglycans). Hence no reliable figures are available concerning the extract's maximum protein concentration either. In the context of possible allergenicity, the size distribution of the protein hydrolysis fragments is important as well. In summary, the VNV Committee concludes that the composition of the product has not been made sufficiently clear.

Production process. The dossier states that the rooster combs used to produce the extract come from poultry (*Gallus gallus*) sourced through licensed abattoirs. The production process begins with enzymatic hydrolysis, which is followed by a number of concentration and desiccation procedures.

The ACNFP makes no safety-related observations regarding the production process or regarding the stability of the extract.

The VNV Committee considers the information provided regarding the production process to be insufficiently detailed to allow the Committee to assess the applicant's assertion that the substances making up the extract are roughly 1% of those making up the rooster combs used as source material. The applicant states that the enzyme used in production is a food-grade enzyme, and supports this claim by referring to certificates held by the producer. However, the VNV Committee does not consider the documents to provide assurance regarding the matter. The applicant goes on to say that, during processing, the product undergoes heat treatment, which inactivates the production enzyme. However, the relevant process conditions are not specified and the summary data on page 23 of the dossier do not adequately demonstrate the effectiveness of the inactivation procedure. Moreover, the VNV Committee does not consider it apparent from the available information whether the inactivated enzyme is removed. Consequently, it is not possible to tell what proportion of the total protein content is accounted for by the enzyme. Careful control of the heat treatment procedure by the applicant is regarded as very important in order to ensure that the powerful enzyme has been completely inactivated.

Estimated intake. The applicant intends to use the rooster combs extract in a variety of foodstuffs, initially including milk, fromage frais, yoghurt and milk-based fermented beverages intended for the general population with the exception of children and pregnant women. The applicant identifies the target group for these products as adults, sportspeople, elderly and menopausal women. The proposal is to add 80 mg of extract per daily portion of

[1] http://en.wikipedia.org/wiki/Glycosaminoglycan

dairy product; the dossier mentions 125 g of yoghurt or 50–100 g of fromage frais as examples of a daily portion (Annex A, Bio11). Use levels for other type of products are not stated. Using data for those EU countries with highest total dairy intake, the applicant has calculated the highest daily consumption of rooster combs extract to be roughly 640 mg per head of the population. This calculation is based upon a total daily dairy intake of roughly 1000 g per person in Finland and Sweden (a figure derived from food availability data (FAOSTAT database)) and assumes that all dairy products consumed contain the novel extract. Using UK food consumption data from 2008 -2009, the applicant also calculated that young children (1-10 years) who eat dairy desserts that contain rooster combs extract despite the fact that the products are not intended for them, could consume an average of 34-38 mg extract per day (Bio11). However, the ACNFP believes that toddlers who particularly like yoghurt and fromage frais might have a much higher level exposure, equating to more than 9 mg of extract per kg bodyweight per day.

The VNV Committee draws attention to recent Dutch food consumption data for various types of dairy products, which is informative in this context (VCP11). The data, obtained from surveys amongst Dutch people between 7 and 69 years old, indicate that, in the various age groups, the 95th percentile of total dairy intake ranges from 721 to 1040 g per day. Generally speaking, yoghurt accounts for at least half of the total dairy intake, even amongst very young children (VCP08). The VNV Committee shares the ACNFP's view that such children are liable to consume a great deal more of the extract than the applicant assumes (Bio11). However, the VNV Committee observes that, without a clear specification of the use levels in the relevant products referred to by the applicant, it is not possible to reliably estimate intake of the novel product.

Previous use. The applicant states that rooster combs are already consumed in the EU, mainly in traditional dishes. Furthermore, a variety of food supplements that contain sodium hyaluronate are available on the European market. However, the only information provided by the applicant regarding the source of such sodium hyaluronate relates to a single product of microbiological origin.

Like the ACNFP, the VNV Committee makes no comment in this context.

Microbiological information. The product specification provided by the applicant includes a number of microbiological parameters; the applicant additionally provided negative test results relating to several production batches. The rooster combs from which the extract is to be obtained will originate from animals certified as suitable for human consumption. The dossier also includes the results of studies performed using model viruses, to illustrate that any viruses potentially present can be effectively inactivated during the production process.

The VNV Committee shares the ACNFP's view that the applicant has demonstrated adequately that the product will be free of relevant species of pathogenic micro-organisms. The assessors were also satisfied that the microbial composition of the yoghurt will not be affected by addition of the novel extract.

Toxicological information. Neither the dossier nor the initial assessment addresses the digestion of rooster combs extract. The applicant does refer to an *in vitro* study of the absorption of the extract through the intestinal mucosa (Table 14), but the results are not included in the dossier. The VNV Committee is not aware of any data on the breakdown of hyaluronate in the gastrointestinal tract.

The applicant has investigated the possibility of the rooster combs extract having toxic effects by performing a number of animal studies and a genotoxicity test with bacteria. Information was also provided regarding a small number of studies into the extract's claimed benefits for horses and people. The findings are summarised in Table 14 of the dossier and incorporated into the initial assessment report. The ACNFP accepts the applicant's assertion that there is no reason to believe that the extract has any adverse effects and regards the highest test dose used in a 90-day study with rats (i.e. 600 mg/kg BW/day) as the NOAEL. The ACNFP has accordingly calculated that the safety margin varies from 54 to 137 for, respectively, the highest and lowest estimated intakes postulated in the applicant's worst case scenario. The ACNFP considers it highly unlikely that the safety margin for toddlers will be less than 100 and therefore has no significant concerns regarding the safety of children who consume rooster combs extract contrary to the applicant's intention.

The VNV Committee agrees with the ACNFP that the available toxicological study results give no cause for concern. The VNV Committee notes, however, that the applicant has submitted only a draft report on the 90-day study with rats. On the basis of the pathology information provided, the study appears to have been comprehensive, but it is not clear why OECD guideline 452 for chronic research has been used in this context. In view of the paucity of safety data, a signed finalised version of this essential study report is a prerequisite for making a definitive assessment.

Allergenicity. The applicant originally proposed that a warning should be included on the label of any foodstuff containing the extract, to the effect that the product is unsuitable for anyone who is allergic to sodium hyaluronate or to avian proteins. The ACNFP fears that labelling as described might unnecessarily limit the choice of consumers with such allergies, because it is highly unlikely that the product would trigger an allergic reaction in someone sensitised to egg proteins.

The VNV Committee does not agree unreservedly with the ACNFP, that a warning "Unsuitable forpeople who are allergic to eggs" would be superfluous. The Committee does not believe that the additional data provided by the applicant (Bio11) conclusively demonstrates the absence of serological cross-reactivity between anti-chicken egg protein IgE and proteins in the extract, because the size and characteristics of the serum pool of patients with egg protein allergy was not clearly stated. In common with the ACNFP, the VNV Committee believes that a warning to consumers with chicken meat allergy (which is considerably less common than egg protein allergy) is justified. The rooster combs extract has not been analysed using sera from people with chicken meat allergy, so cross-reactivity cannot be excluded.

Conclusion

According to the ACNFP, it is unlikely that the use of rooster combs extract in dairy products, as proposed in the dossier, entails a health risk to consumers. Although the VNV Committee has no specific reason to be concerned about the safety of this novel product, the Committee cannot endorse the ACNFP's positive final opinion, because of substantial shortcomings observed in various parts of the dossier. The VNV Committee does not therefore feel able to consent to approval until additional information of adequate quality has been made available.

References

Bio11 Answers to the ACNFP comments on the application for the approval of a Rooster/Cockerel Combs Extract (RCE) as a novel food ingredient. Bioiberica, March 5, 2011.

EG97 Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients. Official Journal of the European Communities 1997; L43: 1-6.

EG97a 97/618/EC. Commission Recommendation of 29 July 1997 concerning the scientific aspects and the presentation of information necessary to support applications for the placing on the market of novel foods and novel food ingredients and the preparation of initial assessment reports under Regulation (EC) No 258/97 of the European Parliament of the Council. Official Journal of the European Communities 1997; L253: 1-36.

VCP08 Dutch National Food Consumption Survey -Young Children 2005/2006, see http://www.rivm.nl/bibliotheek/rapporten/350070001.pdf

VCP11 Dutch National Food Consumption Survey 2007-2010. Diet of children and adults aged 7 to 69 years, see http://www.rivm.nl/bibliotheek/rapporten/350050006.pdf

An overview of surveys in The Netherlands is available from http://www.rivm.nl/Onderwerpen/Onderwerpen/V/Voedselconsumptiepeiling/Overzicht_peilingen

De commissie / The Committee

- Prof. dr. G.J. Mulder, *voorzitter / chairman*
  emeritus hoogleraar toxicologie, Universiteit Leiden
  professor emeritus toxicology, Leiden University
- Prof. dr. C.A.F.M. Bruijnzeel-Koomen, *adviseur / advisor*
  hoogleraar dermatologie/allergologie; UMC Utrecht
  professor of dermatology/allergology; University Medical Centre Utrecht
- Dr. ir. M. Dekker
  levensmiddelentechnoloog; Wageningen Universiteit en Researchcentrum
  food technologist; Wageningen University and Research Centre
- Dr. A.F.M. Kardinaal
  voedingskundige; TNO, Zeist
  nutitional expert; TNO, Zeist
- Dr. ir. E.J. Kok
  toxicoloog; RIKILT Instituut voor Voedselveiligheid, Wageningen
  toxicologist; RIKILT, Institute of Food Safety, Wageningen
- Dr. C.F. van Kreijl
  moleculair-bioloog (gepensioneerd); RIVM Bilthoven
  molecular biologist (retired); National Institute of Public Health and the Environment, Bilthoven
- Dr. F.M. Nagengast
  gastro-enteroloog; UMC St Radboud, Nijmegen
  gastro-enterologist; University Medical Centre St Radboud, Nijmegen
- Dr. ir. J.M.A. van Raaij
  voedingsfysioloog; Wageningen Universiteit en Researchcentrum; RIVM Bilthoven
  food physiologist; Wageningen University and Research Centre; National Institute of Public Health and the Environment, Bilthoven
- Dr. G.J.A. Speijers
  toxicoloog (gepensioneerd); RIVM Bilthoven
  toxicologist (retired); National Institute of Public Health and the Environment, Bilthoven
- Dr. ir. E.H.M. Temme
  voedingskundige; RIVM Bilthoven
  nutritional expert; National Institute of Public Health and the Environment, Bilthoven
- Drs. A.I. Viloria Alebesque, *adviseur / advisor*
  Ministerie van VWS, Den Haag
  Ministry of Health, Welfare and Sport, The Hague
- Dr. R.A. Woutersen
  toxicoloog, toxicologisch patholoog; TNO, Zeist
  toxicologist, toxicologic pathologist; TNO, Zeist

Bureau Nieuwe Voedingsmiddelen, CBG/ Novel Foods Unit, MEB

- Dr. C.M.A. van Rossum, *beoordelaar voedselveiligheid / scientific assessor food safety*
- Dr. ir. M. Rutgers, *beoordelaar voedselveiligheid / scientific assessor food safety*
- Drs. E. van Galen, *hoofd BNV / head NFU*

A Samenvatting van het dossier / Summary of the dossier

Application for the Authorization of the use of a Rooster Combs Extract in Dairy Products under *Regulation (EC) No 258/97 for the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients.*

BIOIBERICA S.A.
Plaça Francesc Macià, 7. 8B
08029 Barcelona
SPAIN

EXECUTIVE SUMMARY

February 2011

EXECUTIVE SUMMARY

BIOIBERICA, S.A. obtains a natural extract from rooster combs, containing glycosaminoglycans, proteins and a high percentage of sodium hyaluronate. Rooster combs have been widely consumed in Europe, being part of several traditional dishes and also being used as a delicacy in many of the high cuisine recipes.

Sodium hyaluronate, the main component of our extract, is a natural substance endogenously found in the intercellular matrix of animal and human connective tissues, as in rooster combs where it is highly concentrated. Sodium hyaluronate is responsible of viscoelastic, lubricating and cushioning properties of joints.

Foods naturally containing sodium hyaluronate are very limited. Only viscera and rooster combs have high amounts of this substance. The maintenance of a varied diet, and also due to cultural habits (not all European countries include rooster combs in their diets), makes difficult to consume these products regularly.

Thus, a good way to make up this lack in sodium hyaluronate could be including a rooster combs extract (RCE) in foods which are daily consumed, like dairy products. Milks or yogurts containing our RCE would supply constant amounts of sodium hyaluronate to our diets, helping our joints to keep in healthy conditions. Also, a dairy product containing the RCE will provide an alternative to the currently marketed food supplements (tablets and capsules which contain high amounts of sodium hyaluronate) and also would be a daily alternative to viscera or rooster combs consumption.

Under *Regulation (EC) No 258/97 of the European Parliament and of the Council of 27th January 1997 concerning novel foods and novel food ingredients* (hereafter referred to as the Novel Foods Regulation), BIOIBERICA's RCE would be considered as novel when added to a dairy foods. This novel status is due to that a dairy food containing this RCE hasn't been exposed to a significant degree to the EU population prior May 1997.

Therefore, in order to support joint health of the general population, BIOIBERICA, S.A. would like to launch its RCE as a novel food ingredient in dairy products. The proposed recommended daily intake of the RCE would be 80 mg per day.

The specifications of the RCE have been well defined according to the results from the analytical controls performed on the product, demonstrating that the manufacture of the product is homogeneous and provides comparable batches at the end of the process.

BIOIBERICA, S.A. has also performed stability studies on the RCE alone and in a yogurt supplemented with the extract. The results demonstrate that the RCE is stable showing no degradation through the studies lapses (43 months for the long term stability study performed with the extract alone, and 1.5 months for the stability study performed on the yogurt supplemented with the extract).

According to the stability study of the RCE contained in a yogurt, the RCE has shown to be stable also when used in acidic food systems as in dairy products. Consequently BIOIBERICA, S.A. proposes the use of its extract in the following dairy products:

- Milk-based fermented beverages (~3-5 pH)
- yogurts (~3-5 pH)
- Milks (6.8 pH)
- *Fromage frais*

The studies conducted in order to examine the potential toxicity of the RCE have demonstrated that the extract is safe ruling out any toxicity associated to the product.

In vitro genotoxicity test has demonstrated that the RCE is not genotoxic. Furthermore, an acute toxicity study performed with our product demonstrated that the Minimum Lethal Dose of the RCE is greater than 2000 mg/kg when administered orally in rats Sprague-Dawley.

Similarly a 2-weeks-dose-range-finding study and a 4-weeks repetitive administration study showed that the repeated oral administration of the extract to rats at a maximum dose of 600 mg/Kg/day did not produce any noteworthy alteration, since neither mortality nor clinical signs were observed.

A subchronic study showed no noteworthy changes after repeated oral administration of the extract to rats, for 13 weeks at dose levels of 5, 55 and 600 mg/kg.

All of these toxicity results establish the NOAEL (No observed adverse effect level) at 600 mg/Kg/day. For a 60 Kg adult this would be equivalent to up to approximately 5.76 g/capita/day of the RCE, according to the study of Reagan-Shaw *et al,* 2007.

In order to confirm the safety of the product, BIOIBERICA, S.A. has also studied the product in other animal models as horses (Carmona *et al*, 2009) where no adverse events were reported during the study neither significant changes were observed in plasma and synovial fluid analysis.

BIOIBERICA, S.A. has also tested the extract in two human trials (Kalman *et al*, 2008 and Martínez-Puig *et al*, 2009), one of them performed in volunteers who took supplemented yogurts containing the extract for 3 months. Neither adverse events were reported during the length of both

studies (two and three months respectively), nor significant changes were observed in the studied parameters.

The excellent safety and toxicity results coming from the abovementioned studies, allowed BIOIBERICA, S.A. to direct a supplemented food with the RCE to people concerned in maintaining their joints healthy.

Typically target groups include adult population, sport people, elderly and menopause women. Based on the predicted intake of dairy products published by FAOSTAT (Food and Agriculture Organization of the United Nations, FAO), BIOIBERICA, S.A. has calculated the predicted intake of the RCE according to the serving sizes presented in *Table 5.*

Assuming a theoretical situation in which all the dairy products consumed would contain the extract, which would rarely be the case, the intake of the extract would vary within different European countries between 0.26 and 0.66 g per person per day, representing in any case a level of intake superior to a 10% of the established NOAEL.

So, according in all the toxicity and safety studies' results and the stability test reports, it is clear then, that our RCE is a safe and stable product which can be added as a novel food ingredient on dairy products such as milk-based fermented beverages, yogurts, milks or *fromage frais*, at the recommended daily intake of 80 mg/day.

B Eerste beoordeling / Initial assessment

ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES

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 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. Hyaluronate is synthesised naturally in the human body. 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 history of 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 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. 51-87 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.

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. The Committee also considered the remote possibility that individuals allergic to chicken meat may be allergic to the proteins in RCE and advised that RCE-containing foods be labelled to reflect this.

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, especially 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's assessment focuses on safety and labelling and does not address any nutrition or health benefits that may be claimed for the novel ingredient or for foods that contain it. Nutrition or health claims may only be made if they are specifically authorised under EU Regulation (EC) No 1924/2006. In the case of Rooster Comb Extract, which is proposed as a dietary source of hyaluronic acid, the Committee notes that this substance is produced endogenously in the

human body, and that EFSA has advised that a cause and effect relationship has not been established between the consumption of hyaluronic acid and the maintenance of normal joints[1];

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.

October 2011

[1] EFSA Journal 2009; 7(9):1266 http://www.efsa.europa.eu/fr/efsajournal/pub/1266.htm