Application for the Authorization of the use of a Rooster Combs Extract in Food Supplements 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

NON-CONFIDENTIAL

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NAME AND ADDRESS OF THE APPLICANT / MANUFACTURER

Headquarters:

BIOIBERICA, S.A.
Plaza Francesc Macià, 7, 8-B
08029 Barcelona
SPAIN
Phone: +34 93 490 49 08
Fax: +34 93 490 97 11
http://www.bioiberica.com

Facilities:

BIOIBERICA, S.A.
Ctra. N-II Km. 680.6. Polígono Industrial “Mas Puigvert”
08389 Palafolls (Barcelona)
SPAIN
Phone: +34 93 765 03 90
Fax: +34 93 765 01 02
http://www.bioiberica.com

2. 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 food supplements. This kind of products, normally presented in dosage forms such as capsules, tablets, pills, sachets of powder or similar forms, containing our RCE would supply constant amounts of sodium hyaluronate to our diets, helping our joints to keep in healthy conditions. Also, a food supplement would also 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 food supplement. This novel status is due to that a food supplement containing this RCE hasn't been exposed to a significant degree to the EU population prior May 1997.

In February 2011 BIOIBERICA S.A. started a novel food ingredient application for RCE to be used in dairy products. BIOIBERICA S.A. made a request to the competent authorities of the United Kingdom. After getting the initial positive assessment report from the United Kingdom and answering the reasoned objections which were raised from the EU Member States, the Commission consulted the European Food Safety Authority (EFSA) asking it to carry out an additional assessment for RCE as food ingredient in accordance with Regulation (EC) No 258/97.

In May 2013 EFSA adopted a Scientific Opinion on the rooster comb extract[1], concluding that RCE is safe under the proposed uses and use levels (dairy products at a maximum dose of 80 mg/daily).

The Scientific EFSA Opinion gave sufficient grounds to establish that RCE in the proposed uses and use levels complies with the criteria laid down in Article 3(1) of Regulation (EC) No 258/97, and therefore the Commission adopted in December 2013 the COMMISSION IMPLEMENTING DECISION of 29 November 2013 authorising the placing on the market of rooster comb extract as a novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council (notified under document C(2013) 8319) (2013/705/EU) [2].

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 food supplements. The proposed recommended daily intake of the RCE would be 80 mg per day.

There are no changes in the product specifications for the proposed novel food ingredient from those previously assessed by EFSA except from that the heavy metals limits have been adapted to Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs; the target population is also the same (the general population, with the exception of pregnant women, children and people with adverse reactions to sodium hyaluronate and/or avian protein). The production process is also the same as well as all other aspects described in the previous application for RCE as Novel Food Ingredient intended to dairy products (History of the source organism; Human Exposure; Nutritional information of the ingredient; Stability studies of the ingredient; Microbiological information of the ingredient and Toxicological information), with the remarkable exception of its intended use, which now is proposed to be extended to food supplements.

BIOIBERICA, S.A. has included in this dossier a stability study performed with a food supplement (in capsule form) containing the RCE. The results demonstrate that the RCE is stable showing no degradation through the studies lapses (36 months for the stability study performed in capsules).

Also, we have performed new anticipated intakes for RCE when included in food supplements. This dossier contains a report from an external food research company (Alimentòmica, Spain) which provides the information about the estimated intake of rooster combs extract (RCE) in Europe when incorporated to food supplements at a level of 80 mg of RCE daily. This report

[1] EFSA Journal 2013;11(6):3260

[2] Official Journal of the European Union 2013; L 322/39

concludes that the exposure to RCE-supplements amongst consumers and the high intake hypothesis of exposure to all potential sources of RCE are quite below the no observed effect level previously considered in the authorization of RCE as a novel food ingredient for dairy products.

So, according to all the toxicity and safety studies previously reviewed by EFSA and the stability test reports, as well as the anticipated intakes, it is clear then that our RCE is a safe and stable product which can be used as a novel ingredient in food supplements at the recommended daily intake of 80 mg/day.

INTRODUCTION

BIOIBERICA S.A. obtains from rooster combs a natural extract, which contains glycosaminoglycans, proteins and a high percentage of sodium hyaluronate.

Sodium hyaluronate is a natural substance endogenously found in the human body and it is responsible of synovial fluid viscoelastic properties, lubricating, cushioning and ensuring a proper function of the joint (smooth, without friction…). It is found in the intercellular matrix of animal connective tissues, such as in rooster combs where it is highly concentrated.

Rooster combs, are nowadays used as part of many dishes in European countries (like France and Spain). It is also used as a typical ingredient in many dishes like homemade chicken soup, stews, several traditional dishes and also used in "haute cuisine" recipes (see Annex V for more examples of the use of rooster combs in nowadays food). It is clear that the rooster combs have an established tradition of safe human consumption in Europe.

BIOIBERICA, S.A. would like to launch its RCE as a novel food ingredient food supplements. This kind of food products containing RCE would help in supplementing our actual diets, lacking of sodium hyaluronate and glycosaminoglycans, due to the decrease in certain animal tissues consumption such as rooster combs.

At the same time, a food product containing this extract would also provide an alternative to the food supplements products currently present in the European market (mostly in Belgium, France, Germany, Ireland, Italy, Portugal, Spain, UK, etc…) which greatly differ on the amounts of sodium hyaluronate.

BIOIBERICA S.A. has prepared this dossier according to *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*.

Section 4 of the Commission Recommendation outlines recommendations made by the Scientific Committee for Food (SCF), which facilitates the safety and nutritional evaluation of a given novel food ingredient. Of the six classes identified, BIOIBERICA's RCE would be allocated at Class 2.1 designed as: *A complex (non-GM derived) novel food ingredient, whose source has a history of food use in the Community.*

This dossier for the Application of Authorization of our RCE as a novel food ingredient follows the Commission Recommendation requirements for the application as follows:

I. Specification of the novel food
II. Effect of the production process applied to the novel food
III. History of the organism used as the source of the novel food
IV-VIII. Not applicable as our RCE is not a GM food.
IX. Anticipated intake of use of the novel food
X. Information from previous human exposure to the novel food or its source
XI. Nutritional information on the novel food
XII. Microbiological information on the novel food
XIII. Toxicological information on the novel food

I. SPECIFICATIONS OF THE NOVEL FOOD

This section contains a general description of the principle components of the RCE, the product specifications and the analytical controls.

BIOIBERICA, S.A. performs batch-to-batch analytical controls in order to make sure that the RCE fulfills the specifications. Section *I.3 Specifications compliance*, contains a table showing the results from the analytical controls performed in ten different batches manufactured during 2012 and 2013. All the analytical reports are enclosed in *Annex I. Analysis Results Reports*.

I.1. GENERAL DESCRIPTION

RCE is the result of the extraction from rooster combs, which have been widely consumed in Europe for years. Nowadays, rooster combs keep on being part of our diets, and are included in products like soups, stews and also as part of sophisticated dishes (See Annex V which contains rooster combs recipes).

RCE consists in a natural extract of rooster combs containing a high percentage of sodium hyaluronate (60-80%), glycosaminoglycans (about 20%) and partially hydrolyzed proteins (about 20%).

SODIUM HYALURONATE, the main component of RCE, is found in the intercellular matrix of animal connective tissues, such as in rooster combs where it is highly concentrated. It was first isolated in 1934 by Karl Meyer from vitreous humour eye. It consists in a linear polysaccharide, whose basic unit is a disaccharide of D-glucuronic acid and N-acetyl-D-glucosamine linked by a glucuronidic (1-3) bond. The disaccharides units are linearly polymerized by hexosaminidic (1-4) linkages.

CAS Number : 9067-32-7

Chemical name : [→4)-O-(β-D-glucopyranosyluronic acid)-(1→3)-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→]

Formula : $[C_{14}H_{20}NNaO_{11}]_n$

Molecular weight : 800,000 Da approximately

Structure :

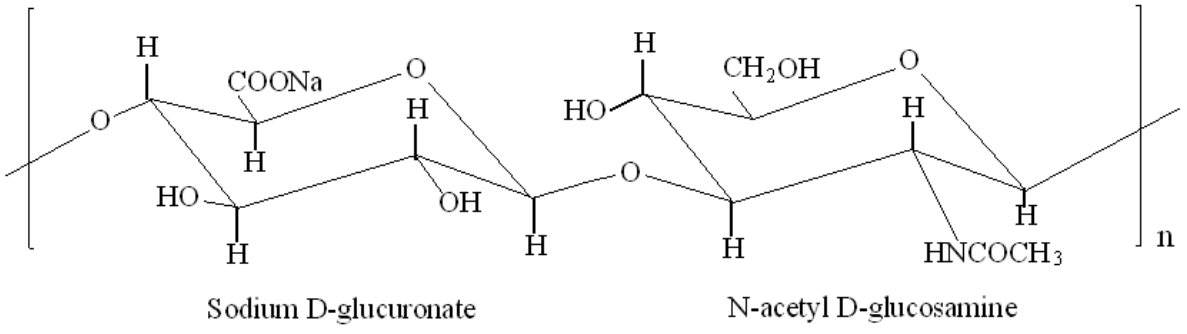

Figure 1. Sodium Hyaluronate molecular structure

GLYCOSAMINOGLYCANS are long unbranched chains of polysaccharides consisting of a repeating disaccharide unit, which form key structural components of the articular cartilage, such as chondroitin sulfate and dermatan sulfate.

Chondroitin sulfate A is a C4-sulphated glycosaminoglycan. Its basic units are disaccharides of D-glucuronic acid and N-acetyl-D-galactosamine linked by a ß-1,3-glycosidic bond; these are polymerized via ß-1,4-glycosidic bonds.

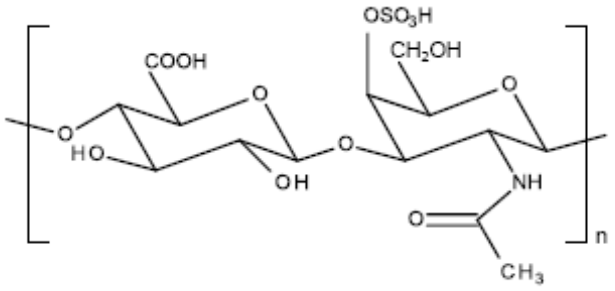

Figure 2. Chondroitin sulfate A molecular structure

In the disaccharide unit of dermatan sulfate (chondroitin sulfate B) glucuronic acid is replaced by the epimeric iduronic acid.

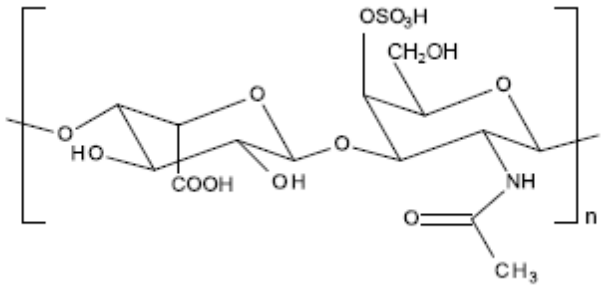

Figure 3. Dermatan sulfate (Chondroitin sulfate B) molecular structure

HYDROLYZED PROTEINS are polypeptides, peptides and amino acids obtained by the hydrolysis of the proteins already present in RCE, such as hydrolyzed collagen.

Other minority components naturally present in the rooster comb and also in the RCE are minerals like sodium, calcium and phosphorous, which can be found in trace amounts.

I.2. PRODUCT SPECIFICATIONS

Product specifications were set according to the analytical results obtained from the very first 3 manufactured batches. Product specifications are shown in *Table 1. Specifications of the RCE.*

SPECIFICATIONS	LIMITS	METHODS
PHYSICO-CHEMICAL PARAMETERS		
Content in glucuronic, expressed as Sodium Hyaluronate	60 – 80 %	Sodium hyaluronate
Hyaluronic Acid	60 – 80 %	Capillary electrophoresis and HPLC
Chondroitin Sulfate (Chondroitin Sulfate – A)	Not more than 5%	HPLC
Dermatan Sulfate (Chondroitin Sulfate – B)	Not more than 25%	Capillary electrophoresis and HPLC
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
Protein by Lowry	Not more than 25 %	Eur. Ph. 2.5.33
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
Cadmium	Not more than 1 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	Environmental Protection Agency (EPA) Method 1613
PCB's	Not more than 4.0 pg/g	
MICROBIOLOGICAL PARAMETERS		
Total viable aerobic count	Not more than 10^3 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

Table 1. Specifications of the RCE

I.3. SPECIFICATIONS COMPLIANCE

Specifications compliance is shown in Tables 2 and 3.

Table 2. RCE analytical results, corresponds to the analysis of 10 different batches of the final product manufactured during 2012 and 2013 including the median and the standard deviation.

As it can be seen on this table, the analytical results for the specification compliance are very stable and homogeneous.

Some parameters like specific heavy metals (mercury, cadmium and lead), dioxins and furans, and PCB's are not analyzed on every single batch. This is due to that the safety and quality of the RCE is well established, so the analysis of these parameters is performed just to assure the absence of these substances once a year.

The external laboratories used for the analysis of RCE specifications parameters are:

- *Institut Químic de Sarrià*
- *Laboratorios Dr. Echevarne*

Both of them are accredited laboratories as it can be seen on the ENAC (National Accreditation Entity) website:

http://www.enac.es/web/enac/busqueda-por-empresa

Moreover, Annex I contains the results of the analysis performed on RCE with a specific method based on capillary electrophoresis (performed in an external laboratory: EUROFINS BIOLAB S.L.U., Barcelona, Spain).

The content of hyaluronic acid was determined (61.7 % as is, not dry matter) and the fractions of Chondroitin sulfate A and Chondroitin sulfate B (Dermatan sulfate) were also quantified (together representing a 13.4 %).

Table 3. Statistics results, contains the results of the mean, the median, the standard deviation and the standard error of all the analytical results obtained from 10 batches manufactured between 2012 and 2013 which are presented in *Table 2. RCE analytical results*.

ANALYTICAL PARAMETERS	SPECIFICATION LIMITS	BATCH ANALYTICAL RESULTS											
		12/0001 Sep 2011	12/0006 Feb 2012	12/0015 Mar 2012	12/0020 Apr 2012	12/0027 Jul 2012	12/0034 Sep 2012	12/0040 Nov 2012	13/0001 Jan 2013	13/0005 Jan 2013	13/0015 Mar 2013	Median	Standard deviation
Sodium Hyaluronate	**60-80 %**	67	66	69	63	66	68	66	65	67	64	66	1,72
Appearance	**White or almost white hygroscopic powder**	Almost white	Almost white	Almost white	Almost white	Almost white	Almost white	Almost white	Almost white	Almost white	Almost white	-	-
pH	**5.0 - 8.5**	6,8	6,7	6,6	7,1	6,9	7,1	6,8	6,6	7	7,5	6,9	0,26
Chlorides (%)	**Not more than 1%**	≤ 1	≤ 1	≤ 1	≤ 1	≤ 1	≤ 1	≤ 1	≤ 1	≤ 1	≤ 1		
Nitrogen (%)	**Not more than 8%**	7	6	7	7	6	6	7	6	6	7	6	0,52
Protein by Lowry (%)	**Not more than 25%**	21,42	-	18,47	-	15,49	-	18,08	-	-	-		
Loss on drying (%)	**Not more than 10%**	6	7	7	6	6	5	8	7	6	5	6	0,90
Heavy metals (ppm)	**Not more than 20 ppm**	≤ 20	-	-	-	-	-	-	≤ 20	-	-	-	-
Mercury (ppm)	**Not more than 0.10 ppm**	≤ 0,10	-	-	-	-	-	-	≤ 0,10	-	-	-	-
Cadmium (ppm)	**Not more than 1 ppm**	≤ 0,50	-	-	-	-	-	-	≤ 0,50	-	-	-	-
Lead (ppm)	**Not more than 0.5 ppm**	≤ 0,50	-	-	-	-	-	-	≤ 0,50	-	-	-	-
Dioxins and Furans (pg WHO/TEQ/g)	**Not more than 2.0 pg/g**	0,02	-	-	-	-	-	-	0,01	-	-	-	-
PCB's (pg WHO/TEQ/g)	**Not more than 4.0 pg/g**	0,003	-	-	-	-	-	-	0,01	-	-	-	-

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ANALYTICAL PARAMETERS	SPECIFICATION LIMITS	BATCH ANALYTICAL RESULTS										Median	Standard deviation
		12/0001 Sep 2011	12/0006 Feb 2012	12/0015 Mar 2012	12/0020 Apr 2012	12/0027 Jul 2012	12/0034 Sep 2012	12/0040 Nov 2012	13/0001 Jan 2013	13/0005 Jan 2013	13/0015 Mar 2013		
Microbiological controls													
Total Aerobic Count (cfu/g)	**Not more than 10^3 cfu/g**	≤ 10^2	≤ 10^2	≤ 10^2	≤ 10^2	≤ 10^2	≤ 10^2	≤ 10^2	≤ 10^2	≤ 10^2	≤ 10^2	-	-
E.coli	**Absence/g**	Absence	Absence	Absence	Absence	Absence	Absence	Absence	Absence	Absence	Absence	-	-
Salmonella	**Absence/ 10 g**	Absence	Absence	Absence	Absence	Absence	Absence	Absence	Absence	Absence	Absence	-	-
Staphylococcus aureus	***1.1.1.1.1.1*** **Absence/g**	Absence	Absence	Absence	Absence	Absence	Absence	Absence	Absence	Absence	Absence	-	-
P.aeruginosa	**Absence/g**	Absence	Absence	Absence	Absence	Absence	Absence	Absence	Absence	Absence	Absence	-	-

Table 2. RCE analytical results

PARAMETERS	SPECIFICATION LIMITS	MEAN	MEDIAN	STANDARD DEVIATION	STANDARD ERROR
Sodium Hyaluronate	60-80 %	66,18%	66.0%	1,72	0,54
pH	5.0 - 8.5	6,91	6,9	0,26	0,08
Nitrogen (%)	Not more than 8%	6,45%	6.0%	0,52	0,17
Loss on drying (%)	Not more than 10%	6,27%	6.0%	0,90	0,29

Table 3. Statistics results

These statistic results show that, even if the product is an extract and this fact could cause a great variability in the analytical results, there is a good homogeneity among the results due to the low value of the standard deviation and also the low value of the standard error in the analyzed parameters.

I.4. ANALYTICAL PROCEDURES FOR THE ROOSTER COMBS EXTRACT

Next table summarizes the information which can be found in *Annex II. Analytical methods*, which are the analytical controls performed on the RCE. Almost all below mentioned analytical methods are official control methods from the European Pharmacopoeia.

PARAMETERS	REFERENCE	TITLE
CHEMICAL METHODS		
Content in glucuronic, expressed as Sodium Hyaluronate	Eur. Ph. Monograph 1472	Sodium hyaluronate
Hyaluronic Acid	Eur. Ph. 2.2.47	Capillary electrophoresis and HPLC
Chondroitin Sulfate (Chondroitin Sulfate – A)	Eur. Ph. 2.2.29	HPLC
Dermatan Sulfate (Chondroitin Sulfate – B)	Eur. Ph. 2.2.47	Capillary electrophoresis and HPLC
pH	Eur. Ph. 2.2.3	Potentiometric determination of pH
Chlorides	-	Mohr Method
Nitrogen	Eur. Ph. 2.5.9	Determination of nitrogen by sulphuric acid digestion
Protein by Lowry	Eur. Ph. 2.5.33	Total Protein
Loss on drying	Eur. Ph. 2.2.32	Loss on drying
Heavy metals	USP <231>	Heavy Metals
Mercury Cadmium Lead	Eur. Ph. 2.2.58	Inductively coupled plasma-mass spectrometry (ICP-MS)
Dioxins and furans	-	Environmental Protection Agency (EPA) Method 1613: Tetra-through octa-chlorinated dioxins and furans by isotope dilution HRGC/HRMS
PCB's	-	
MICROBIOLOGICAL METHODS		
Total viable aerobic count	Eur. Ph. 2.6.12	Microbiological examination of non-sterile products (Total viable aerobic count)
Escherichia coli *Salmonella sp.* *Staphylococcus aureus* *Pseudomonas aeruginosa*	Eur. Ph. 2.6.13	Microbiological examination of non-sterile products (Test for specified micro-organisms)

Table 4. Analytical methods

I.5. ANALYTICAL PROCEDURES FOR THE ROOSTER COMBS EXTRACT IN A FOOD SUPPLEMENT (CAPSULE)

We propose the following official methods for the determination of the presence of RCE in a capsule.

PARAMETERS	REFERENCE	TITLE
CHEMICAL METHODS		
Identification of RCE	UPS <726>	Capillary electrophoresis
Assay of RCE	Eur. Ph. Monograph 1472	Sodium hyaluronate

Table 5. Analytical methods for RCE in a finished product

II. EFFECT OF THE PRODUCTION PROCESS APPLIED TO THE NOVEL FOOD

II.1. DESCRIPTION OF THE MANUFACTURING PROCESS

RCE is obtained by an extraction process from rooster combs, using a mild enzymatic hydrolysis and subsequent filtration, concentration, precipitation and anhydrification. After this process the product will be dried and milled becoming the final white-almost white powder called RCE.

II.1.1. FLOWCHART OF THE ROOSTER COMBS EXTRACT MANUFACTURING PROCESS

II.2. STABILITY OF ROOSTER COMBS EXTRACT

Studies under accelerated storage conditions (40 ± 2ºC / 75 ± 5% Relative Humidity, RH) and long-term storage conditions (25 ± 2ºC / 60 ± 5% RH) have been started with three different production batches of the RCE.

BATCHES TESTED AND CONTAINER CLOSURE SYSTEM:

Three different RCE batches were tested at the two different storage conditions. Each sample was stored in a triple low density polyethylene (LDPE) bag and placed inside a metal drum.

# STUDY	# BATCH	CONDITIONS	DURATION	CONTAINER CHARACTERISTICS
1	5/024	Accelerated Stability	6 months	Triple LDPE bag, closed by cable ties and inside a metal drum
2	6/004	Accelerated Stability	6 months	
3	6/005	Accelerated Stability	6 months	
1	5/024	Long Term Stability	43 months	Triple LDPE bag, closed by cable ties and inside a metal drum
2	6/004	Long Term Stability	40 months	
3	6/005	Long Term Stability	40 months	

TEST AND QUALITY SPECIFICATIONS STUDIED:

The following parameters were selected due to their susceptibility to change with time. These possible changes could affect the quality of the product and, for this reason, they are indicative of the novel food ingredient stability.

TEST	SPECIFICATION
Appearance	White or almost white hygroscopic powder
pH	5.0 – 8.5
Loss on drying	Not more than 10 %
Sodium Hyaluronate	60 – 80 %
Total Viable Aerobic count	Not more than 1000 cfu/g
Moulds and yeasts	Not more than 100 cfu/g
Escherichia coli	Absence/g
Staphylococcus aureus	Absence/g
Salmonella	Absence/g

STUDIES CARRIED OUT:

Accelerated study:

Samples from the three batches to be studied were stored at 40 ± 2ºC / 75 ± 5 % RH in a climatically-controlled chamber. Testing frequency is as follows: 0, 3 and 6 months. As this study is finished, results from the 3-month control are available.

Long-term study:

Samples from the three batches to be studied were stored at 25 ± 2ºC / 60 ± 5 % RH in a climatically-controlled chamber. Testing frequency is as follows: 0, 6, 12, 18, 24, 30, 40 (for batches 6/0004 and 6/0005) and 43 (for batch 5/0024) months. As this study is finished, results from the 40-month and 43-month controls are available.

RESULTS FROM TEST CONTROLS:

Accelerated stability study (40ºC / 75% RH)

Batch 5/024

PARAMETER	INITIAL	3 MONTHS	6 MONTHS
Appearance	Complies	Complies	Complies
pH	6.9	6.4	6.9
Loss on drying (%)	5.6	5.9	5.5
Sodium Hyaluronate (%)	68.0	67.3	65.5
Total Viable Aerobic count	Absence/g	Not determined	Absence/g
Moulds and yeasts	Absence/g	Not determined	Absence/g
Escherichia coli	Absence/g	Not determined	Absence/g
Staphylococcus aureus	Absence/g	Not determined	Absence/g
Salmonella	Absence/g	Not determined	Absence/g

Batch 6/004

PARAMETER	INITIAL	3 MONTHS	6 MONTHS
Appearance	Complies	Complies	Complies
pH	7.1	6.8	6.6
Loss on drying (%)	6.0	6.4	7.3
Sodium Hyaluronate (%)	62.3	61.7	61.0
Total Viable Aerobic count	Absence/g	Not determined	Absence/g
Moulds and yeasts	Absence/g	Not determined	Absence/g
Escherichia coli	Absence/g	Not determined	Absence/g
Staphylococcus aureus	Absence/g	Not determined	Absence/g
Salmonella	Absence/g	Not determined	Absence/g

Batch 6/005

PARAMETER	INITIAL	3 MONTHS	6 MONTHS
Appearance	Complies	Complies	Complies
pH	6.9	6.8	6.8
Loss on drying (%)	5.2	6.0	5.9
Sodium Hyaluronate (%)	61.4	60.0	62.2
Total Viable Aerobic count	Absence/g	Not determined	Absence/g
Moulds and yeasts	Absence/g	Not determined	Absence/g
Escherichia coli	Absence/g	Not determined	Absence/g
Staphylococcus aureus	Absence/g	Not determined	Absence/g
Salmonella	Absence/g	Not determined	Absence/g

Long term stability study (25ºC / 60% RH)

Batch 5/0024

PARAMETER	INITIAL	6	12	18	24	30	43
Appearance	Complies	Complies	Complies	Complies	Complies	Complies	Complies
pH	6.9	6.9	6.9	6.9	6.9	6.3	6.8
Loss on Drying (%)	5.6	6.0	6.4	5.7	6.2	6.2	6.7
Sodium Hyaluronate (%)	68.0	64.3	61.5	62.4	62.1	66.8	63.4
Total Viable Aerobic count	Absence/g	ND	ND	ND	ND	ND	< 10 cfu/g
Moulds/yeasts	Absence/g	ND	ND	ND	ND	ND	< 10 cfu/g
E. coli	Absence/g	ND	ND	ND	ND	ND	Absence/g
S. aureus	Absence/g	ND	ND	ND	ND	ND	Absence/g
Salmonella	Absence/g	ND	ND	ND	ND	ND	Absence/g

ND: Not Determined

Batch 6/004

PARAMETER	INITIAL	6	12	22	30	40
Appearance	Complies	Complies	Complies	Complies	Complies	Complies
pH	7.1	6.8	6.6	6.7	6.7	6.6
Loss on drying (%)	6.0	7.0	7.5	7.4	7.6	6.9
Sodium Hyaluronate (%)	62.3	63.7	63.6	63.1	61.9	62.4
Total Viable Aerobic count	Absence/g	ND	ND	ND	ND	< 10 cfu/g
Moulds and yeasts	Absence/g	ND	ND	ND	ND	< 10 cfu/g
E. coli	Absence/g	ND	ND	ND	ND	Absence/g
S. aureus	Absence/g	ND	ND	ND	ND	Absence/g
Salmonella	Absence/g	ND	ND	ND	ND	Absence/g

ND: Not Determined

Batch 6/005

PARAMETER	INITIAL	6	12	22	30	40
Appearance	Complies	Complies	Complies	Complies	Complies	Complies
pH	6.9	6.8	6.9	6.8	6.7	6.8
Loss on drying (%)	5.2	5.4	5.8	5.6	5.7	5.7
Sodium Hyaluronate (%)	61.4	62.8	62.5	62.3	60.2	62.0
Total Viable Aerobic count	Absence/g	ND	ND	ND	ND	< 10 cfu/g
Moulds and yeasts	Absence/g	ND	ND	ND	ND	< 10 cfu/g
E. coli	Absence/g	ND	ND	ND	ND	Absence/g
S. aureus	Absence/g	ND	ND	ND	ND	Absence/g
Salmonella	Absence/g	ND	ND	ND	ND	Absence/g

ND: Not Determined

INTERPRETATION OF THE STABILITY TEST RESULTS:

Accelerated storage conditions:

The *appearance* of the RCE, aspect and color, did not change after 6 months under accelerated storage conditions for the three tested batches.

The *pH* values maintained between the specifications limits for all three batches and during the whole accelerated stability study.

Loss on drying parameter was softly increased through the time in two of the three batches (6/004, 6/005). However in both cases this parameter remained within the acceptance criteria after 6 months.

No significant changes in *Sodium Hyaluronate* assay have been seen. The Sodium Hyaluronate content has remained inside the specification limits.

Microbiological parameters had excellent results, showing that the product did not develop any microorganism growth during the 6-months accelerated stability study.

Long-term storage conditions:

The *appearance* of the RCE, aspect and color, did not change after 40 and 43 months under long-term storage conditions for the three tested batches.

The *pH* values maintained between the specifications limits for all three batches and during the whole accelerated stability study.

Loss on drying parameter was increased through the time in all three batches. However in all cases this parameter remained within the limits of the specification at the end of the study.

No significant changes in the *Sodium Hyaluronate* content have been seen. The sodium Hyaluronate content have remained inside the specification limits.

All *microbiological parameters* fitted in the specification limits till the end of the study.

CONCLUSIONS:

Exposure to temperatures up to 40ºC ans a relative humidity of 75 % during 6 months, and 25ºC and 60 % of relative humidity during 40-43 months, 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.

II.3. STABILITY OF ROOSTER COMBS EXTRACT IN A FOOD SUPPLEMENT (CAPSULE)

Studies under accelerated storage conditions (40 ± 2ºC / 75 ± 5% Relative Humidity, RH) intermediate storage conditions (30 ± 2ºC / 65 ± 5% RH) and long-term storage conditions (25 ± 2ºC / 60 ± 5% RH) have been performed with the first production batch of a food supplement in form of capsules containing the RCE.

PRIMARY PACKAGING TESTED:

There are two primary packaging tested:

Packaging 1: Al-PVC/PVDC blister, 12 capsules per blister.

Packaging 2: HDPE white bottle (Duma Special 4350), LDPE cap (Duma handy 4014), silica gel capsule (1.5g). 30 capsules per bottle.

TEST AND QUALITY SPECIFICATIONS STUDIED:

The following parameters were selected due to their susceptibility to change with time. These possible changes could affect the quality of the product and, for this reason, they are indicative of the novel food ingredient stability.

TEST	RELEASE SPECIFICATION	STABILITY SPECIFICATION
Appearance	Anonymous hard capsules, red color. Content: white, or almost white, powder.	Anonymous hard capsules, red color. Content: white, or almost white, powder.
Uniformity of mass	According to Eur. Ph.	According to Eur. Ph.
Average mass (full capsule) mg/capsule	Informative	Informative
Average mass (Content)	237.5 – 262.5 mg/capsule	Informative
Loss on drying (content)	Not more than 8 %	Not more than 14 %
RCE assay	Not less than 36 mg/capsule	Not less than 34 mg/capsule
Total aerobic microbial count(*)	Not more than 10000 cfu/g	Not more than 10000 cfu/g
Total yeast and moulds count(*)	Not more than 100 cfu/g	Not more than 100 cfu/g
Escherichia coli (*)	Absence/g	Absence/g

(*) only will be performed at the last check point.

STUDIES CARRIED OUT:

Packaging 1: Al-PVC/PVDC blister, 12 capsules per blister.

Accelerated stability study:

Samples from the batch to be studied were stored at 40 ± 2ºC / 75 ± 5 % RH in a climatically-controlled chamber. Testing frequency is as follows: 0, 3 and 6 months. As this study is finished, results from the 6-month control are available.

Intermediate stability study:

Samples from the batch to be studied were stored at 30 ± 2ºC / 65 ± 5% RH in a climatically-controlled chamber. Testing frequency is as follows: 0, 3, 12, 18, 24 and 36 months. As this study is finished, results from the 36-month control are available.

Long-term study:

Samples from the batch to be studied were stored at 25 ± 2ºC / 60 ± 5 % RH in a climatically-controlled chamber. Testing frequency is as follows: 0, 3, 9, 12 and 36 months. As this study is finished, results from the 36-month controls are available.

RESULTS FROM TEST CONTROLS:

Accelerated stability study (40ºC / 75% RH)

Batch B01

PARAMETER	INITIAL	3 MONTHS	6 MONTHS
Appearance	Complies	Complies (1)	Complies (2)
Uniformity of mass	Complies	Complies	Complies
Average mass (full capsule)	347.0 mg	356.3 mg	354.8 mg
Average mass (content)	250.0 mg	255.8 mg	256.0 mg
Loss on drying (content)	5.6 %	8.4 %	8.8 %
RCE assay	44.9 mg	48.63 mg	49.3 mg
Microbiological testing	Complies	Not determined	Complies

(1) There is a very slight dimming of the content, almost imperceptible.

(2) Very slight dimming.

Intermediate stability study (30ºC / 65% RH)

Batch B01

PARAMETER	INITIAL	3 MONTHS	12 MONTHS	18 MONTHS	24 MONTHS	36 MONTHS
Appearance	Complies	Complies	Complies	Complies	Complies	Complies
Uniformity of mass	Complies	Complies	Complies	Complies	Complies	Complies
Average mass (full capsule)	347.0 mg	351.1 mg	353.7 mg	353.8 mg	354.1 mg	352.0 mg
Average mass (content)	250.0 mg	253.5 mg	254.9 mg	255.4 mg	254.9 mg	254.5 mg
Loss on drying (content)	5.6 %	7.1 %	7.6 %	7.9 %	7.9 %	4.9 %
RCE assay	44.9 mg	48.4 mg	45.3 mg	43.5 mg	46.4 mg	57.9 mg
Microbiological testing	Complies	Not determined	Complies	Not determined	Not determined	Complies

Long term stability study (25ºC / 60% RH)

Batch B01

PARAMETER	INITIAL	3 MONTHS	9 MONTHS	12 MONTHS	36 MONTHS
Appearance	Complies	Complies	Complies	Complies	Complies
Uniformity of mass	Complies	Complies	Complies	Complies	Complies
Average mass (full capsule)	347.0 mg	346.2 mg	351.7 mg	352.3 mg	356.0 mg
Average mass (content)	250.0 mg	250.2 mg	253.4 mg	254.8 mg	256.1 mg
Loss on drying (content)	5.6 %	6.6 %	7.1 %	7.5 %	4.7 %
RCE assay	44.9 mg	47.3 mg	46.5 mg	44.4 mg	52.7 mg
Microbiological testing	Complies	Not determined	Complies	Complies	Complies

Packaging 2: HDPE white bottle (Duma Special 4350), LDPE cap (Duma handy 4014), silica gel capsule (1.5g). 30 capsules per bottle.

Accelerated stability study:

Samples from the batch to be studied were stored at 40 ± 2ºC / 75 ± 5 % RH in a climatically-controlled chamber. Testing frequency is as follows: 0, 3 and 6 months. As this study is finished, results from the 6-month control are available.

Intermediate stability study:

Samples from the batch to be studied were stored at 30 ± 2ºC / 65 ± 5% RH in a climatically-controlled chamber. Testing frequency is as follows: 0, 3, 12, 18, 24 and 36 months. As this study is finished, results from the 36-month control are available.

Long-term study:

Samples from the batch to be studied were stored at 25 ± 2ºC / 60 ± 5 % RH in a climatically-controlled chamber. Testing frequency is as follows: 0, 3, 9, 12 and 36 months. As this study is finished, results from the 36-month controls are available.

RESULTS FROM TEST CONTROLS:

Accelerated stability study (40ºC / 75% RH)

Batch B01

PARAMETER	INITIAL	3 MONTHS	6 MONTHS
Appearance	Complies	Complies	Complies
Uniformity of mass	Complies	Complies	Complies
Average mass (full capsule)	347.0 mg	349.3 mg	350.5 mg
Average mass (content)	250.0 mg	253.4 mg	254.3 mg
Loss on drying (content)	5.6 %	7.0 %	7.2 %
RCE assay	44.9 mg	49.7 mg	51.3 mg
Microbiological testing	Complies	Not determined	Complies

Intermediate stability study (30ºC / 65% RH)

Batch B01

PARAMETER	INITIAL	3 MONTHS	12 MONTHS	18 MONTHS	24 MONTHS	36 MONTHS
Appearance	Complies	Complies	Complies	Complies	Complies	Complies
Uniformity of mass	Complies	Complies	Complies	Complies	Complies	Complies
Average mass (full capsule)	347.0 mg	348.3 mg	350.7 mg	350.8 mg	352.1 mg	354.0 mg
Average mass (content)	250.0 mg	250.9 mg	253.9 mg	253.2 mg	254.5 mg	255.9 mg
Loss on drying (content)	5.6 %	6.5 %	6.9 %	7.3 %	7.3 %	4.8 %
RCE assay	44.9 mg	43.2 mg	46.1 mg	43.1 mg	46.0 mg	41.5 mg
Microbiological testing	Complies	Not determined	Complies	Not determined	Not determined	Complies

Long term stability study (25ºC / 60% RH)

Batch B01

PARAMETER	INITIAL	3 MONTHS	9 MONTHS	12 MONTHS	36 MONTHS
Appearance	Complies	Complies	Complies	Complies	Complies
Uniformity of mass	Complies	Complies	Complies	Complies	Complies
Average mass (full capsule)	347.0 mg	347.8 mg	348.9 mg	348.8 mg	351.5 mg
Average mass (content)	250.0 mg	250.1 mg	251.8 mg	252.3 mg	253.9 mg
Loss on drying (content)	5.6 %	6.2 %	6.3 %	6.6 %	4.3 %
RCE assay	44.9 mg	45.4 mg	45.7 mg	44.4 mg	51.0 mg
Microbiological testing	Complies	Not determined	Complies	Complies	Complies

INTERPRETATION OF THE STABILITY TEST RESULTS:

All parameters analyzed remained between the specification limits, also the content of RCE, which remained stable through the time at different assayed conditions.

CONCLUSIONS:

Exposure to temperatures up to 40ºC and a relative humidity of 75 % during 6 months, 30ºC and a relative humidity of 65 % during 36 months and 25ºC and 60 % of relative humidity during 36 months, using two kinds of packaging did not compromise the stability of the final product neither of the RCE.

III. HISTORY OF THE ORGANISM USED AS THE SOURCE OF THE NOVEL FOOD

The novel food ingredient is obtained from an edible non-GM biological source (rooster combs). The source organism is fully characterized and this and/or the food obtained from it are not detrimental to human health.

Rooster combs has had an established human consumption in Europe for ages and continues to be part of the normal diet, including frequently consumed dishes such as home-made recipes (stews) and industrially prepared soup concentrates. First evidences of the use of rooster combs are found in medieval recipes books from 15th century.

Furthermore, rooster combs are also still used as a delicacy in restaurants. See Annex V for more information.

III.1. ROOSTER COMBS EXTRACT SOURCE

Our RCE, is an animal origin product obtained from rooster (*Gallus gallus*) combs declared as fit for human consumption.

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 and weight more than 8 gr.

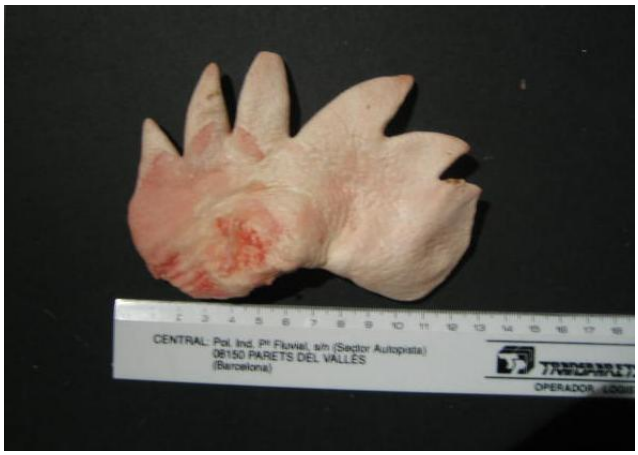

Image 1. Rooster comb

Evidence of the use of rooster combs goes back to medieval recipes in the 15th century, according to the recipes book Platina's On Right Pleasure and Good Health, 1468. (Milham, 1998)

Nowadays, we can find rooster combs as part of our diets incorporated as an ingredient in home-made chicken soup and also in several traditional dishes. In some countries like Spain or France, rooster combs are commonly used as a delicacy (See *Annex V. Rooster combs recipes*), based on an established tradition of consumption in these countries.

Also, culinary recipes can be easily found on the internet.

Packed rooster combs (*Crestas de gallo de corral* in spanish) can also be found as for example in the Spanish Market as shown in the picture below.

Therefore, sodium hyaluronate, glycosaminoglycans and proteins present in rooster combs have been consumed directly by individuals for ages.

Image 2. Packed rooster combs from the Spanish market.
Source: http://www.tiendaargi.es/index.php?main_page=product_info&products_id=16

IX. ANTICIPATED INTAKE OF USE OF THE NOVEL FOOD

RCE could be incorporated in many different food matrices (as the authorized use in dairy products) as a novel food ingredient. However, in this case we propose RCE to be incorporated in any possible form of food supplements (capsules, tablets, pills, sachets of powder or similar forms).

The food supplement containing RCE will be taken at a maximum recommended dose of 80 mg of the RCE per day (i.e. 1 capsule daily containing 80 mg of RCE or 2 capsules daily containing 40 mg of RCE).

IX.1. PROPOSED USE GROUPS FOR THE USE OF ROOSTER COMBS EXTRACT

The RCE is proposed for the use in food supplements for the general population with the exception of pregnant women, children and people allergic to sodium hyaluronate and/or avian proteins.

It is expected that the product would be consumed by adult population, sport people, elderly, and menopause women. The final product containing RCE will be destined to maintain joint health of healthy population with joint discomfort.

Considerations on pregnant women and children

Even it can be deduced from human data we already have that no potentially toxicity risk should be expected when RCE is administered to pregnant women and children, no toxicity studies are yet available for these concrete segment of the population. Due to that, the final product will be labeled for conscious consumers and will contain warnings for pregnant women and children.

Considerations on allergenicity

No allergic episodes have been described in the human and animal studies as a result of the RCE supplementation. As stated in section I.1, RCE contains sodium hyaluronate (60-80%), glycosaminoglycans (about 20%) and partially hydrolyzed proteins (about 20%). Both sodium hyaluronate and glycosaminoglycans have a broad history of use among the UE market (as oral food supplements) without any documented adverse report related to allergenicity.

In addition, is biologically plausible that RCE has an extremely reduced allergenic potential as deduced by different aspects:

- The protein fraction of RCE (mainly collagen) is derived from conjunctive tissue which is known to be less allergenic than egg proteins. The absence of egg-white proteins such as ovalbumin (OVA), ovomucoid (OM), lysozyme (LYS) and ovotransferrin (OT) has been confirmed by analysis
- The protein fraction of the RCE is hydrolysed (mean molecular weight 1233.7 ± 6.23 Da). Although on the basis of the current knowledge, the degree of hydrolysis cannot

fully predict the immunogenic or the allergic potential of a protein[3], it is considered that a protein is more likely to have allergic potential when the molecular weight is higher than around 3000 Da[4].

In any case, BIOIBÉRICA, S.A. has tested RCE for its capacity of binding serum IgE from egg allergic patients by indirect inhibition ELISA. Sera obtained to test the egg protein allergenicity of the Rooster Combs Extract (RCE) were collected by authorized personnel. The participants were always monitored with appropriate medical assistance and signed a written consent for the collection of the sera. The study center (the Food Science Research (CIAL) of the Spanish National Research Council (CSIC)) also had the authorization from the corresponding bioethics committee.

The results obtained from this test demonstrated that none of the three batches of RCE showed any binding capacity to IgE from a pooled serum of patients with egg allergy (see *Annex VI. Allergenicity Report*).

However, theoretically it could be some cases of hypersensitivity to sodium hyaluronate or avian proteins. Thus, it will also be reasonable to include a warning in the label of the final product containing the RCE for people allergic to sodium hyaluronate and/or avian proteins.

[3] Høst A, *et al*. *Dietary products used in infants for treatment and prevention of food allergy. Joint statement of the European Society for Paediatric Allergology and Clinical Immunology (ESPACI) Committee on Hypoallergenic Formulas and the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) Committee on Nutrition*. Arch Dis Child 1999;81:80-84

[4] Van Beresteijn ECH, *et al*. *Molecular mass distribution, immunological properties and nutritive value of whey protein hydrolysates*. J Food Protect 1994;57:619–625.

IX.2. INDIVIDUAL PROPOSED FOOD USES AND USE LEVELS

A daily dose of 80 mg of the RCE per capita and per day is the recommended individual intake established, according to the efficacy studies, in order to appropriately nourishing the joints.

Toxicity studies performed by BIOIBERICA S.A. (see a summary in point *XIII.2. Toxicity studies performed with the RCE*; original copies are enclosed in Annex VII) have shown that the recommended dose of the extract (80 mg/ day) is completely safe.

The historical intake of the original source of the extract, rooster combs, also shows that the recommended dose is safe. The proposed amount of 80 mg daily corresponds to a remarkably inferior quantity of the extract compounds present in a meal portion of rooster combs.

RCE's compounds are present in a comb at an approximate proportion of 1%. So, 25 g of rooster combs (considering a meal portion of 3 combs of approximately 8 g per comb), contain 250 mg of the product, a quantity which is noticeably superior to the recommended daily dose (80 mg).

IX.3. PREDICTED INTAKES

Annex VIII of this dossier contains a report from an external food research company (Alimentòmica, Spain) which provides the information about the estimated intake of rooster combs extract (RCE) in Europe when incorporated to food supplements at the level of 80 mg of RCE daily.

Using The European Food Safety Authority (EFSA) Comprehensive Database, this report remarks that:

The “high intake scenario” (sum of estimated mean intake from four dairy food sources previously authorized, as milks, yogurts, etc… plus the highest 95th percentile intake which was estimated for food supplements) for “consumers only”, the highest daily intake would occur in adults in Italy (1.971 g). The highest intake scenario for “all subjects” was estimated for elderly in Finland (0.792 g/day).

In its previous opinion on RCE the EFSA-NDA-Panel noted that this type of intake methodology for fortified foods is generally considered to be “high intake”, as a result of conservative assumptions made in the consumption estimates where it is assumed that all food products within a food category contain the ingredient at the maximum specified level of use (EFSA 2013).

However, this report concludes that the exposure to food supplements containing RCE amongst consumers and the high intake hypothesis of exposure to all potential sources of RCE are quite below the no observed effect level previously considered in the authorization of the other uses (dairy products).

IX.4. PREDICTED INTAKES FOR RISK GROUPS

At the recommended doses, no risk group has been studied.

A food supplement containing the RCE is not addressed to any risk group as pregnant women or children.

X. INFORMATION FROM PREVIOUS HUMAN EXPOSURE TO THE NOVEL FOOD OR ITS SOURCE

Human exposure to the source (Rooster combs):

As already commented, rooster combs, have been widely used in the Community as a food ingredient. Annex V of this dossier contains some of the evidences of its use in Europe.

As RCE's compounds are present in a comb at an approximate 1%, the recommended daily dose (80 mg / day) is inferior to the quantity of the RCE's compounds (250 mg) we can naturally find in a normal portion of combs (25 g, considering 3 combs of 8 g each).

Human exposure to the ingredient (Sodium Hyaluronate):

European market is full of products in form of food supplements, containing this ingredient. These products are sold with no adverse effects reports (See section *X.2 Previous exposure to Sodium Hyaluronate preparations*).

Human exposure to the novel ingredient (RCE):

BIOIBERICA, S.A. has performed trials in humans with the proposed novel ingredient where no adverse effects have been observed (See section *XIII.2. Toxicity studies performed with the RCE*).

Therefore, there is plenty of information demonstrating that exposure to the novel food is unlikely to give rise to nutritional, microbiological, toxicological and/or allergenicity problems.

X.1. PREVIOUS ROOSTER COMBS INTAKE

As commented in section *III.1 Rooster combs extract source*, there are lots of evidences which show that rooster combs have been consumed directly by individuals in Europe for years. Therefore, safety of the source of RCE is demonstrated.

It would not be expected that the mild enzymatic digestion, concentration and precipitation to which the rooster combs are subjected would alter the safety of sodium hyaluronate, glycosaminoglycans and hydrolyzed proteins obtained in the extract.

In order to demonstrate the safety of its product, BIOIBERICA, S.A. has performed some toxicological studies, administering intraperitoneal and orally the RCE to rats. Moreover, many other studies demonstrate that no adverse effects, neither allergenicity reactions were observed when studying the efficacy of the RCE administered orally to horses and humans. Section *XIII Toxicological information on the novel food* contains all safety evidences for the RCE.

X.2. PREVIOUS EXPOSURE TO SODIUM HYALURONATE PREPARATIONS

Some examples of the high quantity of food supplements (tablets / capsules) containing Sodium Hyaluronate, present in the European market (mostly Belgium, France, Germany, Ireland, Italy, Portugal, Spain, and UK), are summarized in the following table. Some other product examples are widely explained in Annex IX of this dossier.

BRAND NAME	mg OF SH* PER CAPSULE / TABLET	NUMBER OF CAPSULES / TABLETS RECOMMENDED PER DAY	PRODUCT FORM	SOLD ON
Vital-HA	150 mg	3 capsules (450 mg of SH)	Capsules	On-line
Ultimate HA Formula	150 mg	3 capsules (300 mg of SH)	Capsules	On-line
Synovoderma	210 mg	3 – 6 capsules (from 630 to 1,260 mg of SH)	Capsules	On-line
Puritan's Pride Hyaluronic Acid	100 mg	1 – 4 capsules (from 50 to 200 mg of SH)	Capsules	On-line
Vit.O.Best	36 mg	2 – 4 capsules (from 72 to 144 mg of SH)	Capsules	On-line
Solaray Hyaluronic Acid	60 mg	1 capsule (60 mg of SH)	Capsules	On-line
Hydra-Plenish	100 mg	2 – 4 capsules (from 200 to 400 mg of SH)	Capsules	On-line
Pure HA	100 mg	2 capsules (200 mg of SH)	Capsules	On-line
Skin Eternal Hyaluronic Acid	100 mg	2 tablets (200 mg of SH)	Tablets	On-line
Natrol Hyaluronic Acid	100 mg	4 tablets (400 mg of SH)	Tablets	On-line
Source Natural Hyaluronic Acid	100 mg	2 tablets (200 mg of SH)	Tablets	On-line
Richelet® Anti-Age Peau existe	2.2 mg	1 tablet (2.2 mg of SH)	Tablets	On-line
Hyaluronic Forte®	120 mg	1 tablet (120 mg of SH)	Tablets	On-line

Table 7. Food supplements marketed in the European Union containing sodium hyaluronate

*SH: sodium hyaluronate

As stated in the previous table, Sodium Hyaluronate recommended doses vary from one supplement to another. Almost all of the food supplements containing sodium hyaluronate and present in the market can be easily obtained from the internet. Also, most of supplements do not specify the source of its sodium hyaluronate except “Hydra-Plenish” and “Solaray Hyaluronic Acid” which its source is microbial fermentation.

Our proposal is to include RCE in food supplements at a recommended maximum dose of 80 mg/day, a lower dose than the majority of the example products nowadays present in the market.

XI. NUTRITIONAL INFORMATION ON THE NOVEL FOOD

RCE in food supplements is not intended to replace any food. Nowadays, consumption of rooster combs has been decreased and, as a consequence, also the Sodium Hyaluronate intake. A food supplement containing the RCE would be a good supplementation to our diets. BIOIBERICA, S.A. has performed a trial in humans consuming food supplements with the RCE (See section *XIII.4. Human Studies*).

Next tables show the nutritional information of the RCE itself and the nutritional values when the RCE is added to a food supplement in form of capsule.

NUTRITIONAL INFORMATION OF THE RCE		
PARAMETERS	**PER 100 g**	**PER RECOMMENDED DAILY DOSE (80mg)**
Energy	23.01 kcal / 100 g 95.9 KJ / 100 g	0.02 kcal 0.07 KJ
Carbohydrates (by difference)	35.6 g	0.03 g
Fats	0.16 g	0 g
Protein (Kjeldahl Nitrogen x 6.25)	43.81 g	0.03 g
Crude Fiber (Weende method)	<0.5 g	0 g
Sodium	2.86 g	0 g

Table 8 . RCE nutritional information

As an example, we have included 40 mg of RCE in a food supplement in form of capsules. 2 capsules daily should be taken to achieve the maximum recommended dose for RCE (80 mg/day).

This example capsule containing RCE could have the following composition:

Next table summarizes the nutritional information of this capsule containing RCE:

NUTRITIONAL INFORMATION OF A FOOD SUPPLEMENT WITH RCE		
PARAMETERS	**PER 100 g**	**PER RECOMMENDED DAILY DOSE (2 capsules)**
Energy	242 kcal / 100 g 990 KJ / 100 g	1.68 kcal 6.85 KJ
Protein (Kjeldahl Nitrogen x 6.25)	23.7 g	0,16 g
Non-protein nitrogen	1.15 g	0,01 g
Fats	0.78 g	0,01 g
Carbohydrates (by difference)	0.44 g	0 g
Total Fiber (AOAC 985.29)	69.4 g	0,48 g
Crude Ash	2.4 g	0,02 g

Table 10. Nutritional information of a food supplement containing RCE

XII. MICROBIOLOGICAL INFORMATION ON THE NOVEL FOOD

The manufacturing extraction process of the RCE is based in the Good Manufacturing Practices and moreover, BIOIBERICA, S.A. controls the presence of any microorganism (virus and bacteria) which could alter the quality and safety of the product.

Table 1 of section *I.2 Product Specifications,* summarizes all parameters controlled and the methods used. Appropriate limits for total viable aerobic count and reference pathogen micro-organisms are established and complied with.

Microbiological controls are conducted on a batch-to-batch basis in order to guarantee that the manufacturing process is capable to eliminate any potential bacterial contamination present in the raw material and to prevent bacterial growth during production.

RCE is obtained from animals declared fit for human consumption (See Health Certificate attached in Annex III). Careful selection of this animal raw material provides a measure of confidence that the final product is safe as regards potential viral contamination. Nevertheless, the manufacturing process is performed under specific conditions that are efficient in the inactivation/removal of several conventional viruses.

Annex X contains the final report of a study which evaluates the clearance of MLV, influenza, REO3 and PPV by the purification process for the RCE for the ability to eliminate adventitious and endogenous virus contaminants. This study was performed by BioReliance Invitrogen Bioservices, in September 2006, and designed to comply with the CPMP Note for Guidance on Virus (models) Validation Studies. The study concludes that different steps of the purification process of our RCE, concretely the enzymatic digestion, solvent precipitation and H_2O_2 treatment, were effective in the inactivation/removal of viruses considered as models.

As for a food supplement containing the RCE, section *II.2 Stability of the RCE in a food supplement,* contains the microbiological controls 36 months after the study started, demonstrating that the addition of the RCE to a food supplement does not promote the presence of any pathogenic microorganism.

XIII. TOXICOLOGICAL INFORMATION ON THE NOVEL FOOD

BIOIBERICA, S.A. has studied the safety and efficacy of the proposed novel food ingredient in various different trials (See section *XIII.2. Toxicity studies performed with the RCE, section XIII.3. Other animal studies with the RCE and section XIII.4. Human studies.*

There is no information suggesting that the novel food might pose allergenic risk to humans. None of the trials performed in humans or in animals have reported any adverse effect or allergenic reactions.

However, BIOIBÉRICA, S.A. in order to make sure about the non-allergenic risk of RCE has performed and allergenic test in an external Research center as mentioned in *Section IX.1. Proposed use groups for the use of Rooster Combs Extract.*

Annex VI of this dossier contains the report on “RESEARCH ON EGG PROTEIN ALLERGENICITY IN ROOSTER COMBS EXTRACT”.

XIII.1. TOXICOLOGICAL ASSESSMENT

RCE is a demonstrated safe product. Many analytical and toxicity studies show its harmlessness.

Next three tables summarize the analytical results of the presence of heavy metals, dioxins, furans and PCB's in the RCE:

HEAVY METALS ANALYSIS

RCE BATCH	MERCURY	CADMIUM	LEAD
11/0001	< 0.10 ppm	< 0.50 ppm	< 0.50 ppm
12/0001	< 0.10 ppm	< 0.50 ppm	< 0.50 ppm
13/0001	< 0.10 ppm	< 0.50 ppm	< 0.50 ppm

Table 11. Analytical results for heavy metals

DIOXINS AND FURANS ANALYSIS

RCE BATCH	pg WHO / TEQ / g
11/0001	0.02
12/0001	0.02
13/0001	0.01

Table 12. Analytical results for dioxins and furans

PCBs ANALYSIS

RCE BATCH	pg WHO / TEQ / g
11/0001	0.005
12/0001	0.003
13/0001	0.01

Table 13. Analytical results for PCB's

XIII.2. TOXICITY STUDIES PERFORMED WITH THE ROOSTER COMBS EXTRACT

Next table contains a summary of the toxicity studies performed with the RCE in rats. Moreover, we have included, as a support, some efficacy studies of the product in horses and in humans, showing the safety and the harnessless of the product. The last study included in the table below was performed in humans, studying the safety and efficacy of our RCE as an ingredient in yogurts, so in the matrix where we would like to commercialize our product. According to all the results from the toxicity studies conducted with the RCE, and the history of safe use of its raw material (rooster combs), we can state that our RCE is a safe novel ingredient.

TITLE OF THE STUDY	TYPE	SUBJECT STUDIED	ADMIN. ROUTE	DOSIFICATION	SAFETY CONCLUSIONS
Genotoxicity test	In vitro	*Salmonella E.Coli*	-	Five concentrations	• No toxicity in any of the strains • No mutagenic responses
Acute oral toxicity in rat	In vivo	18 rats	Orally (gastric gavage)	1,000 mg/Kg 2,000 mg/Kg	• No mortality at 2000 mg/Kg • No clinical signs during or after treatment
2-week dose-range-finding study	In vivo	40 rats	Orally (gastric gavage)	200 mg/Kg/day 400 mg/Kg/day 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	Orally (gastric gavage)	5 mg/Kg/day 55 mg/Kg/day 600 mg/Kg/day	• No mortality neither alterations in feed consumption, 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	Orally (gastric gavage)	5 mg/Kg/day 55 mg/Kg/day 600 mg/Kg/day	• No mortality neither alterations in feed consumption, body weight or necropsies • No clinical or histological signs observed.

This table continues on the next page

TITLE OF THE STUDY	TYPE	SUBJECT STUDIED	ADMIN. ROUTE	DOSIFICATION	SAFETY CONCLUSIONS
Acute intraperitoneal toxicity in rat	In vivo	26 rats	Intraperitoneally	250 mg/Kg/day 500 mg/Kg/day 900 mg/Kg/day 1000 mg/Kg/day	• No mortality observed. • Observed clinical signs post administration as abnormal locomotion, piloerection. • Minimum Lethal Dose of the RCE established in more than 1000 mg/Kg
Study of the intestinal absorption of the RCE	In vitro	6 rats	-	Solution of 200 µg/ml of the RCE	• 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	Orally	250 mg/day	• Treated horses presented higher levels of hyaluronate in the synovial fluid. Any adverse reaction was observed or any clinical sign. No lameness neither joint effusion occurred.
Clinical Trial on efficacy and safety of the RCE. (8 weeks administration)	In vivo	20 adults	Orally	80 mg/day	• No serious adverse events were reported. • The RCE appeared to be well tolerated and safe. • No alteration son body weight, vital signs, and safety laboratory results.
Clinical trial evaluating the efficacy and safety of a yogurt supplemented with the RCE	In vivo	40 adults	Orally	80 mg/day	• No significant changes in body weight or clinical parameters as pulse rate or blood pressure were observed.

Table 14. Summary of all Toxicity and Efficacy studies performed with the RCE

XIII.2.1. GENOTOXICITY TEST

This bacterial reverse mutation study was carried out by RCC CIDA S.A. in Barcelona, Spain. The study was performed following Good Laboratory Practices (GLP) according to the OECD Principles of Good Laboratory Practice and Directive 2004/10/EC.

The study begun in January 2008 and the final report (No.S11430) was signed in July 2008. See Annex VII for the complete study.

STRAINS USED

Ames strains TA-1535, TA-1537, TA-98 and TA-100 of *Salmonella typhimurium* and *Escherichia coli* WP2 uvra pkM101 were treated with BIOIBERICA's RCE, at five different concentrations. The aim of this study was to assess the possible mutagenic potential of the RCE in presence and absence of a metabolic activation system.

PROCEDURES

Bacterial preparation:

Five bacterial strains were defrosted and grown on Master plates in order to obtain pure cultures. These plates were stored between 2 and 8 ºC. Sixteen hours before each test, an inoculums was prepared for each of the five bacterial strains in 20 ml of nutrient broth and was incubated in a bath a 37 ± 1 ºC with agitation.

Test item preparation:

Dimethylformamide was used as a solvent for the test item (RCE). 100 mg of the extract was wighed and dimethylformamide was gradually added.

Metabolic activation system preparation:

Homogenized rat liver (S-9), induced with Aroclor was used to check whether the test item was active when metabolized. S-9 was used at a concentration of 10% in standard cofactors.

Experimental procedure:

0.1 ml of test item or the positive control were preincubated with 0.1 ml of the strain culture and 0.5 ml of sterile phosphate buffer or of metabolic activation system for 20 minutes at 37 ± 1ºC. Plates were incubated for 72 hours at 37 ± 1ºC. After the incubation period, the number of

relevant colonies that had grown on each plate was counted. The whole experiment was repeated another day, using fresh solutions and fresh bacterial cultures.

Positive control:

Parallel to the study of the test item, known mutagenic products were tested in order to check the sensitivity of mutagenic agents of the strains used.

Negative control:

The solvent used to dilute the test item, dimethylformamide, was tested as the negative control.

STATISTICAL ANALYSIS

The comparisons between the results for the reference item and the control were made using Student's *t* test with levels of significance of $p < 0.01$ and $p < 0.05$. The statistical comparison of the different test-item concentrations and the control was carried out, for all the bacterial strains, both with and without metabolic activation, using a one-way analysis of variance with a level of significance of $p < 0.05$.

RESULTS

- All bacterial strains responded positively when treated with the positive controls. Accordingly, normal values were obtained in the revertant colony counts on all the plates treated only with the solvents (negative controls).
- About the test item, the RCE did not cause toxicity in any of the strains. No mutagenic response was observed in any of the tested strains, with or without the S-9.

CONCLUSIONS

- Based on the results, it may be concluded that the test item RCE, under the experimental conditions used produced no mutagenic activity in any of the five bacterial strains used.

XIII.2.2. ACUTE ORAL TOXICITY IN RAT

The Acute Oral Toxicity test on the RCE was carried out by the *Centro de Investigación y Desarrollo Aplicado, S.A.L.* (CIDASAL) in Barcelona, Spain. The study was performed following Good Laboratory Practices (GLP) according to the OECD Principles of Good Laboratory Practice and Directive 1999/11/EC.

The study was begun in March 2004 and the final report (No. CD04/9053T) was signed in May 2004. See Annex VII for the complete study.

ANIMALS USED

18 rats Sprague Dawley “SD” (9 male and 9 female) were used in the study. The animals in the Preliminary Study weighed between 110-141 g and those used in the Principal Study weighed 137–150 g and 119–131 g for the male and female rats, respectively.

The rats were housed in Makrolon cages (59.0 x 38.5 x 20.0 cm) in groups of maximum 5 rats of the same gender during the period of acclimatation. The cages were identified by means of a card indicating the number of the study and the housed animals, their sex, code of the tested product, dose, administration route, date of administration, date of entrance of the animals and person in charge of the study. The cages were distributed on the shelves in order to equilibrate any external factor (environmental conditions) that could have an influence on the animals.

The study was performed at a temperature of 20-25ºC, and a relative humidity of 30-65%.

The illumination was artificial and controlled to provide 12 hours of light (7:00 to 19:00 h) and 12 hours of darkness.

DIET

The animals had free access to a standard diet for rats SAFE A04C.

WATER

The animals had water *ad libitum* provided with bottles. The water is analyzed periodically to detect the presence of potential contaminants.

ROUTE OF ADMINISTRATION, FREQUENCY AND VOLUME

The test product, the RCE codified as IB0004, was administered orally by means of a gastric sonde. The administration was carried out in one time, with a volume of 10 ml/kg for the dose of 1000 mg/kg. The dose of 2000 mg/kg was administered fractionated, allowing a period of 4 hours between the first and second administration, with a volume of 20 ml/kg.

The volume of administration to each animal was determined according its weight at the moment of administration.

The rats administered with the dose of 1000 mg/kg were fasted for about 18 hours before the administration, and the rats administered with the dose of 2000 mg/kg for 12-16 hours.

All the animals were offered food 3-4 hours after the treatment.

DOSE LEVEL AND GROUP SIZE

Preliminary Study

The RCE was administered as follows:

TREATMENT	DOSE (mg/Kg)	No ASSIGNED TO THE ANIMALS	
		Male	Female
RCE (IB0004)	1000	1, 2	3, 4
	2000	5, 6	7, 8

Principal Study

The RCE was administered as follows:

TREATMENT	DOSE (mg/Kg)	No ASSIGNED TO THE ANIMALS	
		Male	Female
RCE (IB0004)	2000	9 to 13	14 to 18

OBSERVATIONS

Preliminary Study

After the administration, the rats were observed at least twice a day for 14 days. After this period, the animals were sacrificed.

Principal Study

During the day of the administration, the rats were observed frequently, in order to detect clinical signs. Additionally, the animals were observed at least twice a day for 14 days.

The observations included, at least, changes in the skin, hair, eyes and mucous membranes, in the respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behavioral pattern.

After this period of observation, the animals were sacrificed.

Body weight

All the rats were weighed before the administration and at half of the period of observation. Before their sacrifice, the animals were again weighed.

Sacrifice and *post-mortem* procedures

After the period of observation, all the animals were sacrificed by intraperitoneal injection of sodium pentobarbital, performing the necropsy of all the animals from the Principal Study.

The necropsy included the assessment of the intact animal and all of the superficial tissues, followed by the observation of the cranial, thoracic and abdominal cavities.

RESULTS

Preliminary Study

- None of the animals administered with the RCE at a dose of 1000 and 2000 mg/kg died.
- No clinical signs were observed in the same animals neither during the day of the administration nor during the days following the treatment.
- The evolution of the corporal weight of all the animals was normal.

Principal Study

- None of the animals administered with the RCE at a dose of 2000 mg/kg died.
- No clinical signs were observed in any animal treated with a dose of 2000 mg/kg, neither during the day of the administration nor during the days following the treatment.
- The evolution of the corporal weight of all the animals was normal.
- One male and one female showed a dark area in the stomach wall of about 1 and 5 mm of diameter, respectively. The rest of the necropsys didn't show macroscopic alterations.

CONCLUSION

- No mortality was observed at the highest dose administered, 2000 mg/kg. This dose is determined by the maximum concentration of the product soluble in distilled water that allows a formulation suitable for its oral administration in rats at the maximum volume of administration of 20 ml/kg.
- Consequently, considering the dose of 2000 mg/kg high enough, the Maximum Non-Lethal Dose and the Minimum Lethal Dose, were not determined.
- According to the results obtained, the Minimum Lethal Dose of the RCE is greater than 2000 mg/kg when administered orally in rats Sprague Dawley.

XIII.2.3. 2-WEEK DOSE-RANGE-FINDING STUDY

This test on the RCE was carried out by the Centro de Investigación y Desarrollo aplicado, S.A.L. (CIDASAL) in Barcelona, Spain. The study was performed following the Good Laboratory Practices (GLP) according to the OECD Principles of Good Laboratory Practice and Directive 2004/10/EC.

The study was begun in November 2004 and the final report (No. CD04/9438T) was signed in February 2005. See Annex VII for the complete study.

ANIMALS USED

40 rats Wistar Hannover HsdBrlHan:WIST (20 males and 20 females) were used in the study. Medium weight of the animals was 134g for males, and 123 g for females.

The animals were housed in Makrolon cages (59.0 x 38.5 x 20.0 cm) in groups of maximum 5 rats of the same gender. Each cage was identified by a card, colour-coded according to the dose level. This card indicated the number of the cage, number and sex of the animals housed, number of the study, test item code, administration route, dose, name of the Study Director, and date of arrival of the animal and of the start of treatment.

The study was performed at a temperature of 22 ± 3 ºC and a relative humidity of 40-75%.

The lighting in the animal housing was controlled to give 12 hours of light (7:00 to 19:00) and 12 hours of darkness (19:00 to 7:00) every 24 hours.

The cages were arranged on the shelving so that external factors, such as environmental conditions, were balanced as much as possible.

DIET

The animals had free access to a standard rodent diet SAFE A04C.

WATER

The animals had water *ad libitum* provided with bottles. The water was analyzed periodically to detect the presence of potential contaminants.

ROUTE OF ADMINISTRATION, FREQUENCY AND VOLUME

The test item, the RCE (IB0004), was administered orally by gastric gavage.

The animals were administered once a day, seven days a week, during 2 weeks with a volume of 20 ml/Kg.

The volume of administration to each animal was determined according its weight at the moment of administration.

The rats from the Control group were treated only with the vehicle (distilled water), at the same administration volume as the rest of the treatment groups.

DOSE LEVELS AND GROUP SIZE

The study was conducted with four groups, including the control group, and rats were distributed in the following way:

GROUP		1	2	3	4
Tested product		Control	IB0004	IB0004	IB0004
Dose (mg/kg/day)		-	200	400	600
Colored code		White	Blue	Green	Red
Number	Males	1 to 5	6 to 10	11 to 15	16 to 20
	Females	21 to 25	26 to 30	31 to 35	36 to 40

- Doses:

- Due to the characteristics of the test item, the dose levels were selected based on the maximum dose administrable by oral route (600 mg/kg day).

OBSERVATIONS

Mortality

Two rats found dead during the study underwent the necropsy procedure (see Sacrifice and macroscopic examination).

Clinical signs

All rats were observed in great detail at least twice a day, in order to record any symptom of illness or reaction to the treatment.

The observations, also made at the weekends, included at least, changes in the skin, fur, eyes and mucous membranes, in the respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behavioral.

Body weight

The body weight of each rat was recorded before the start of treatment, daily during the treatment period, and before sacrifice.

Food intake

The food intake per cage was recorded before the start of treatment, and then once a week after that, and the mean weekly intake per rat was calculated.

SACRIFICE AND MACROSCOPIC EXAMINATION

At the end of the second week of treatment, all rats were sacrificed by means of exsanguination after deep anesthesia with pentobarbital sodium by intraperitoneal route.

A full autopsy was carried out on all animals. This included an examination of the external surface of the body, all orifices, cranial, thoracic and abdominal cavities, and their contents both *in situ* and after evisceration.

Following the macroscopic examination, some organs were weighed and some other organ samples were taken for a histological examination.

STATISTICAL ANALYSIS

The body weights and organs weights were evaluated by means of a one-factor analysis of variance. When statistically significant differences were found, the differences among groups were evaluated using Dunnett's method ($p<0.05$ and $p<0.01$).

RESULTS

- No mortality or clinical signs attributable to the treatment among the animals treated with the RCE.
- Body weight of the animals treated with the RCE and the animals of the Control group had no alterations along the period of treatment.
- Food intake among the animals treated with the RCE wasn't affected by the treatment, and it was similar to the Control group. The exception was the group administered at 200 mg/kg/day that had a higher food intake than the Control group, but this happened even at the beginning of the treatment.
- Necropsies of the sacrificed animals, showed only in some animals treated at doses of 400 mg/kg/day and 600 mg/kg/day, some reddish coloring on the thymus and on the mandibular lymph nodes. There were no gross lesions that could be attributed to the test item. All findings recorded were considered to be within the range of normal background lesion, which may be seen in rats of this strain.
- No other macroscopic alterations were observed in necropsies, neither differences in the organ weight among the animals treated with the RCE.

CONCLUSION

- The repeated administration of the test item, the RCE, to rats during a period of two consecutive weeks, at the doses of 200, 400 and 600 mg/Kg/day did not produce any noteworthy alteration, since neither mortality or clinical signs were observed and there were no differences in body weights in comparison with Control group.
- The only thing observed was a reddish colouring on the mandibular lymph nodes and reddish areas on the thymus, in some animals from the high and intermediate doses.
- Based on the results obtained, the dose of 600 mg/kg/day is proposed as the high dose in the 4-week study, since it was the maximum dose that could be administered by oral route.

XIII.2.4. ORAL TOXICITY BY 4-WEEKS REPETITIVE ADMINISTRATION

The Oral Toxicity test on the RCE was carried out by the Centro de Investigación y Desarrollo aplicado, S.A.L. (CIDASAL) in Barcelona, Spain. The study was performed following the Good Laboratory Practices (GLP) according to the OECD Principles of Good Laboratory Practice and Directive 2004/10/EC.

The study was begun in March 2005 and the final report (No. CD04/9491T) was signed January 2006. See Annex VII for the complete study.

ANIMALS USED

100 rats Wistar Hannover HsdBrlHan:WIST (50 males and 50 females) were used in the study. Medium weight of the animals was 103 g for males, and 99 g for females.

The animals were housed in Makrolon cages (59.0 x 38.5 x 20.0 cm) in groups of maximum 5 rats of the same gender. Each cage was identified by a card, colour-coded according to the dose level. This card indicated the number of the cage, number and sex of the animals housed, number of the study, test item code, administration route, dose, name of the Study Director, and date of arrival of the animal and of the start of treatment.

The cages were arranged on the shelving so that external factors, such as environmental conditions, were balanced as far as possible.

The study was performed at a temperature of 22 ± 2 ºC and a relative humidity of 30-60%.

The lighting in the animal housing was controlled to give 12 hours of light (7:00 to 19:00) and 12 hours of darkness (19:00 to 7:00) every 24 hours.

DIET

The animals had free access to a standard rodent diet SAFE A04C.

WATER

The animals had water *ad libitum* provided with bottles. The water was analyzed periodically to detect the presence of potential contaminants.

ROUTE OF ADMINISTRATION, FREQUENCY AND VOLUME

The tested product, the RCE, was administered orally by gastric gavage.

The administration was carried out once a day, seven days a week, during 4 weeks with a volume of 20 ml/Kg.

The volume of administration to each animal was determined daily according its weight at the moment of administration.

The rats from the Control group were treated only with the vehicle (distilled water), at the same administration volume as the rest of the treatment groups.

DOSE LEVEL AND GROUP SIZE

The study was performed with four groups, including the control group, and rats were distributed in the following way:

GROUP		1	2	3	4
Tested product		Control	RCE	RCE	RCE
Dose (mg/kg/day)		-	5	55	600
Colored code		White	Blue	Green	Red
Sacrifice at the end of treatment	Males	1 to 10	16 to 25	26 to 35	36 to 45
	Females	51 to 60	66 to 75	76 to 85	86 to 95
Sacrifice after recovery period	Males	11 to 15	-	-	46 to 50
	Females	61 to 65			96 to 100

- Doses:
  - High dose of 600 mg/Kg/day was selected to be the maximum oral dose administrated due to the solubility characteristics of the product.
  - Low dose of 5 mg/Kg/day was selected as a lower multiple of the foreseen dose for humans (0.66 mg/Kg/day approximately).

- Intermediate dose of 55 mg/Kg/day was selected as a geometric medium dose between the maximum and the minimum doses.

RECOVERY PERIOD

Once concluded the period of treatment, 5 males and 5 females from control group and from the maximum dose treatment group, were subjected to a 2 weeks recovery period where the animals didn't have any administration.

The objective of this recovery period was to study the reversibility or the evolution of possible alterations registered.

OBSERVATIONS

Mortality:

All rats were observed daily just to detect clear signs of morbidity or mortality.

Clinical signs:

All rats were observed in great detail at least once a day, in order to record any symptom of illness or reaction to the treatment.

The observations included at least, changes in the skin, fur, eyes and mucous membranes, in the respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behavioral.

Body weight:

The body weight of each rat was recorded before the start of treatment, daily during the treatment period, and before sacrifice.

Those rats subjected to the 2 weeks recovery period were weighed twice a week.

Food intake

The food intake per cage was recorded one week before the start of treatment, and then once a week after that, and the mean weekly intake per rat was calculated.

Ophthalmoscopy:

The eyes of all the animals were examined before the beginning of the treatment.

During the last week of treatment, the eyes of the animals belonging to the control group and to the maximum dose group were examined.

Later, during the last week of the recovery period, the eyes of the animals belonging to these groups were examined again.

The examination was carried out by means of eye drops of cyclopentolate chlorohydrate at 1% in order to dilate the pupils.

Laboratory studies:

During the fourth week of treatment, blood samples were extracted from 10 males and 10 females of each group. These animals didn't have any access to food during 16 hours previous to the extraction.

Blood samples of each animal were extracted between 7:30 and 10:00 am, in order to reduce the biological variation caused by circadian rhythms.

Also, urine samples were collected during 16 hours, of the same 10 males and 10 females of each group.

Later, during the last week of the recovery period blood and urine samples of all animals subjected to this recovery period were analyzed.

SACRIFICE AND MACROSCOPIC EXAMINATION

By the end of the fourth week of treatment, all rats were sacrificed by means of exsanguination after deep anesthesia with pentobarbital sodium by intraperitoneal route, except from those rats subjected to the recovery period that were sacrificed at the end of this.

A full autopsy was carried out on all animals. This included and examination of the external surface of the body, all orifices, cranial, thoracic and abdominal cavities, and their contents both *in situ* and after evisceration.

Following the macroscopic examination, some organs were weighed and some other organ samples were taken for a histological examination.

HISTOLOGICAL EXAMINATION

The aim of the microscopic observations was to examine organs and tissues from those animals that some adverse event was observed:

- Tissues from dead animals, in order to find out the cause of the death.
- Tissues from all the animals belonging to the Control group and to the maximum dose group that were sacrificed at the end of the treatment.
- All the organs and tissues of the animals sacrificed at the end of the recovery period from those animals that some effect was observed in the treatment group.

STATISTICAL ANALYSIS

The body weights and organs weights were evaluated by means of a one-factor analysis of variance ANOVA ($p<0.05$ and $p<0.01$). When statistically significant differences were found, the differences among groups were evaluated using Dunnett's method ($p<0.05$ and $p<0.01$).

The rest of urinary parameters were statistically evaluated with an homogeneity test (Chi2 $<p<0.05$).

RESULTS

- No mortality and clinical signs attributable to the treatment among the animals treated with the RCE.
- Body weight of the animals treated with the RCE and the animals of the Control group had no alterations along the period of treatment.
- Food intake among the animals treated with the RCE had no differences from the Control group during the treatment period neither during the recovery period.
- There were no ocular alterations related to the treatment.
- There weren't any alterations attributed to the treatment in the hematological and urinary analysis during the fourth week of treatment, neither during the recovery period.
- No macroscopic alterations attributed to the treatment were observed in necropsies neither at the end of the treatment, nor at the end of the recovery period between the animals treated with the RCE.
- No significant alterations were observed on the organs weight, only a decrease of thymus weight among the females treated at a dose of 600 mg/kg/day.

- There were neither injuries in the histological examination related to the administration of the RCE among the animals sacrificed after the period of treatment, nor after the recovery period.

CONCLUSION

- The repeated administration of the test item, the RCE, in rats during four consecutive weeks, at doses of 5, 55 and 600 mg/Kg/day did not produce any noteworthy alteration, since neither mortality or clinical signs were observed in any of the treatment groups.
- Histological examination of the animals treated with the RCE showed no alterations attributed to the treatment.
- According to the results obtained, the NOAEL (No Observable Adverse Effect Level) may be established at 600 mg/Kg/day for the oral administration to rats during a period of 4 weeks.

XIII.2.5. 13-WEEK ORAL (GAVAGE) TOXICITY STUDY IN RATS WITH A 4-WEEK RECOVERY PERIOD

The Sub-chronic Oral Toxicity test was carried out by Harlan Laboratories S.A. in Barcelona, Spain. The study was performed following the Good Laboratory Practices (GLP) according to the OECD Principles of Good Laboratory Practice and Directive 2004/10/EC.

The study was begun in June 2008 and the final report (No.S11970) was signed in June 2009. See Annex VII for the complete study.

ANIMALS USED

100 rats Wistar Hannover HsdBrlHan:WIST (50 males and 50 females) were used in the study. Body weight range of the animals was 205.2 – 250.4 g for males, and 148.9-182.0 g for females and age of both genders was between 7-9 weeks.

The animals were housed in Makrolon type-5 cages with sawdust bedding Lignocel 3-4, in groups of maximum 5 rats of the same gender. Each cage was identified by a card, and a tail or ear tattoo mark. The study was performed at optimum hygienic conditions behind a barrier system, air-conditioned with a minimum of 10-15 air changes per hour, and continuously monitored environment with target for temperature approximately 22 ± 3 ºC and 12 hours fluorescent light/12 hours of dark.

The group identification and animal numbers assigned to treatment are stated in the following table:

GROUP	1	2	3	4
Tested product	Control	RCE	RCE	RCE
Dose (mg/kg/day)	0 mg/kg/day*	5 mg/kg/day	55 mg/kg/day	600 mg/kg/day
Males A	1-10	16-25	26-35	36-45
Males B**	11-15	-	-	46-50
Females A	51-60	66-75	76-85	86-95
Females B**	61-65	-	-	96-100

A: Main Study (termination after 13 weeks of treatment).
B: Recovery (termination after 13 week of treatment and 4 weeks of recovery).
*: Control animals were treated with vehicle only.
**: According to *OECD Guideline for the Testing of Chemicals Guideline 452 Chronic Toxicity Studies, 12 May 1981*, two satellite groups are included to monitor the reversibility of any toxicological changes induced by the chemical under investigation (control and higher dose).

DIET

Standard dry pelleted diet for rodents Harlan Teklad 2014C rat/mouse maintenance diet *at libitum*. The composition of each batch is analyzed as well as checked for contaminants.

WATER

The animals had access *ad libitum* to community tap water provided by Compañía de Aguas Sabadell S.A.. The water is analyzed periodically to detect the presence of potential contaminants.

ROUTE OF ADMINISTRATION, FREQUENCY AND VOLUME

The tested product, BIOIBERICA's RCE, was administered orally by gastric savage. The administration was carried out once a day, seven days a week, during 13 weeks with a volume of 20 ml/Kg. The rats from the Control group were treated only with the vehicle (distilled water), at the same administration volume as the rest of the treatment groups.

DOSE LEVEL AND GROUP SIZE

Dose level was based on the results obtained from a 4-week toxicity (gavage) study with the test item in rats (*4-week toxicity study in rats with repeated oral administration and a 2-week recovery period*). The study was performed with four groups, including the control group, and rats were distributed in the following way:

GROUP	1	2	3	4
Tested product	Control	RCE	RCE	RCE
Dose (mg/kg/day)	-	5 mg/kg	55 mg/kg	600 mg/kg

- <u>Doses:</u>
  - The high dose (600 mg/kg) was selected because it is the maximum administrable dose by oral route.
  - The low dose (5 mg/kg) was selected because it is a low multiple of the intended human dose (approximately 0.66 mg/kg).

- The intermediate dose was selected because it is the geometric mean between the high and low doses.

RECOVERY PERIOD

Once concluded the period of treatment, 5 males and 5 females from group 1 and 4 underwent a 4-week treatment-free period. This recovery period corresponded to weeks 14 and 17, inclusive, of the Study, considering the first week of treatment as the first week of the Study.

The objective of this recovery period was to study the reversibility or the alterations observed during the treatment period.

OBSERVATIONS

Viability / Mortality

All rats were observed daily just to detect clear signs of morbidity or mortality. Moribund animals and those presenting severe clinical signs were sacrificed for ethical reasons and to avoid autolysis of the tissues.

Clinical signs

All rats were observed in great detail at least once a day, during acclimatization and recovery.

The observations included at least: changes in the skin, fur, eyes and mucous membranes, in the respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behavioral.

Body weight

The body weight of each rat was recorded during the week before of the start of treatment, twice weekly during the treatment weeks 1 to 13 and weekly afterwards, and before sacrifice.

Those rats subjected to the recovery period were weighed weekly. The mean body weights per group and sex were calculated twice a week from the individual weights.

Food intake

Before the beginning treatment, and then once a week during the treatment period and during the recovery period, the food intake per cage was recorded and the weekly mean intake per rat was calculated.

Ophthalmoscopy

These examinations included the cornea, crystalline lens, conjunctivae, sclera, iris and fundus. During each examination, the pupils were dilated. Examinations were taken before the beginning of the study and at the end.

Laboratory studies

Blood samples were extracted from the retro-orbital plexus from all animals. The animals were fasted in metabolism cages for approximately 16-18 hours before blood sampling but allowed access to water *ad libitum*. The samples were collected early in the working day to reduce biological variation caused by circadian rhythms. Urine was also collected during the 16 to 18 hour fasting period.

SACRIFICE AND MACROSCOPIC EXAMINATION

At the end of the treatment all surviving rats were deprived from food for 18 hours before the sacrifice. Then they were deeply anaesthetized with sodium pentobarbital administered itraperitoneally and then exsanguinated by excision of the axillary vessels and aorta, except for those undergoing recovery, which were sacrificed at the end of that period.

Because the total number of animals exceeds the number that could be sacrificed in one day, the necropsies were carried out on several consecutive days. Tissue and organs samples were collected from all animals at necropsy and fixed in neutral phosphate buffered 4% formaldehyde solution.

HISTOLOGICAL EXAMINATION

Slides and all organs and tissues which were collected at terminal sacrifice from the animals of the control and high-dose groups were examined by the study pathologist.

STATISTICAL ANALYSIS

- The Dunnett-test (many to one t-test) based on a pooled variance estimate was applied for the comparison of the treated groups and the control groups for each sex when the variables were assumed to follow a normal distribution.
- The Steel-test (many-one rank test) was applied instead of the Dunnett-test when the data were not assumed to follow a normal distribution.
- Fisher's exact-test was applied to the ophthalmoscopy and macroscopic findings.
- Armitage/Cochran Trend Test was applied for non-neoplastic lesions, when appropriate.
- Student´s t-test was applied to some parameters analyzed during the recovery period.

RESULTS

- No mortality attributable to the treatment among the animals treated with the RCE.
- No noteworthy clinical signs were observed. Just during the treatment period, salivation was observed occasionally, after the administration in some animals of the animals at the three treatment groups (both sexes).
- Food intake among the animals treated with the RCE had no differences from the Control group during the treatment period neither during the recovery period.
- Among males, no statistically significant changes were observed about body weight gain. Among females, statistically significant differences were observed occasionally in body-weight gain among the females treated at 55 mg/kg on days 22, 64, 71, 75 and 82 of the treatment. The mean body weight and body-weight gain (%) in the course of the recovery period , both sexes, was similar to that observed in Control group.
- There were no ocular alterations related to the treatment.
- No changes of toxicological relevance were observed. Statistically significant differences with respect to the Control group were observed in some treated groups in a few parameters: higher mean corpuscular hemoglobin index (MCH), and lower reticulocyte count in males treated at 5 mg/kg, higher mean corpuscular volume (MCV) in males treated at 55 mg/kg and higher platelet values in females treated at 600 mg/kg. However, these differences were devoid of any toxicological significance. At the end of recovery period, no hematological differences were observed in the analyses performed.
- There were no gross lesions that could be attributed to test-item treatment. All findings were considered to be within the range of normal background lesions that may be seen in

rats of this strain and age. The necropsies carried out at the end of the recovery period revealed no noteworthy alterations.

- At the end of treatment period, no variations in organ weight were observed in any of the treatments groups of males. Among the females, the relative organ/weight of liver at the doses of 5 and 600 mg/kg was statistically higher to that recorded in the Control group.
- There were no gross lesions that could be attributed to treatment with test item. The microscopic examination revealed some findings that were considered to be within the range of normal background lesions that may be seen in rats of this strain and age.
- No changes were observed in blood biochemistry parameters in the analyses performed during week 13 of treatment. At the end of recovery period lower statistically significant differences with respect to the Control groups were observed in phosphorous and sodium values. Moreover these differences were fortuitous in nature.

CONCLUSION

- No noteworthy changes were observed after repeated oral administration of the test item, RCE, to rats for 13 weeks at dose levels of 5, 55 and 600 mg/kg.
- Few changes such lower body-weight gain, were observed occasionally in females treated at 55 mg/kg. Furthermore several changes in the hematological blood analyses were observed in all the treatment groups. However, these differences were devoid of any toxicological relevance.
- At the end of treatment period, some differences observed in females liver weight at the doses of 5 and 600 mg/kg could be attributed to the lower weight observed in Control females liver weight.
- No treatment-related histopathological alterations were observed at any of the administered doses.
- The changes observed in the hematological parameters at the end of treatment period are considered not of toxicological relevance and within the range of normal values.
- The changes in sodium and phosphorus levels observed at the end of recovery period were fortuitous in nature and considered within the range of variations recorded for these parameters. Furthermore no noteworthy alterations were observed in the necropsies performed.
- According to the results obtained, the NOAEL (No Observable Adverse Effect Level) may be established at 600 mg/Kg/day for the oral administration to rats during a period of 13 weeks.

XIII.2.6. ACUTE INTRAPERITONEAL TOXICITY IN RAT

The Acute Intraperitoneal Toxicity test on the RCE was carried out by the *Centro de Investigación y Desarrollo Aplicado, S.A.L.* (CIDASAL) in Barcelona, Spain. The study was performed following Good Laboratory Practices (GLP) according to the OECD Principles of Good Laboratory Practice and Directive 1999/11/EC.

The study was begun in March 2004 and the final report (No. CD04/9054T) was signed in June 2004. See Annex VII for the complete study.

ANIMALS USED

26 rats Sprague Dawley "SD" (13 male and 13 female) were used in the study. The animals used in the Preliminary Study weighed between 125-153 g and those used in the Principal Study weighed 150–159 g and 135–149 g for the male and female rats, respectively.

The rats were housed in Makrolon cages (59.0 x 38.5 x 20.0 cm) in groups of maximum 6 rats of the same gender during the period of acclimatation. The cages were identified by means of a card indicating the number of the study and the housed animals, their sex, code of the tested product, dose, administration route, date of administration, date of entrance of the animals and person in charge of the study. The cages were distributed on the shelves in order to equilibrate any external factor (environmental conditions) that could have an influence on the animals.

The study was performed at a temperature of 20-25ºC, and a relative humidity of 30-65%.

The illumination was artificial and controlled to provide 12 hours of light (7:00 to 19:00 h) and 12 hours of darkness.

DIET

The animals had free access to a standard diet for rats SAFE A04C.

WATER

The animals had water *ad libitum* provided with bottles. The water is analyzed periodically to detect the presence of potential contaminants.

ROUTE OF ADMINISTRATION, FREQUENCY AND VOLUME

The test product, the RCE codified as IB0004, was administered intraperitoneally by means of a graduated syringe provided with a needle 25G (0.5 x 16 mm), 23G (0.6 x 25 mm) or 20 G (0.9 x 25 mm) depending on the concentration of the administered solution.

The administration was carried out in one time, with a volume of 10 ml/kg for the dose of 250 mg/kg and with a volume of 20 ml/kg for the rest of the doses. The injection lasted about 0.1 ml/second.

The volume of administration to each animal was determined according its weight at the moment of administration.

DOSE AND SIZE OF THE GROUPS

Preliminary Study

The RCE was administered as follows:

TREATMENT	DOSE (MG/KG)	No ASSIGNED TO THE ANIMALS	
		Male	Female
RCE (IB0004)	250	1, 2	3, 4
	500	5, 6	7, 8
	900	9, 10	11, 12
	1000	13, 14	15, 16

Principal Study

The RCE was administered as follows:

TREATMENT	DOSE (MG/KG)	No ASSIGNED TO THE ANIMALS	
		Male	Female
RCE (IB0004)	1000	17 to 21	22 to 26

OBSERVATIONS

Preliminary Study

After the administration, the rats were observed at least twice a day for 14 days. After this period, the animals were sacrificed.

Principal Study

During the day of the administration, the rats were observed frequently, in order to detect clinical signs. Additionally, the animals were observed at least twice a day for 14 days.

The observations included, at least, changes in the skin, hair, eyes and mucous membranes, in the respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behavioral pattern.

After this period of observation, the animals were sacrificed.

Corporal weight

All the rats were weighed before the administration and at half of the period of observation. Before their sacrifice, the animals were again weighed.

Sacrifice and *post-mortem* procedures

After the period of observation, all the animals were sacrificed by intraperitoneal injection of sodium pentobarbital, performing the necropsy of all the animals from the Principal Study.

The necropsy included the assessment of the intact animal and all of the superficial tissues, followed by the observation of the cranial, thoracic and abdominal cavities.

RESULTS

Preliminary Study

- None of the animals administered with the RCE at the doses of 250, 500, 900 and 1000 mg/kg died.

- Clinical signs in several animals were observed during the first days post administration: abnormal locomotion, hunched posture, piloerection, muscle relaxation and enlarged abdomen.
- The evolution of the corporal weight of all the animals was normal.

Principal Study

- None of the animals administered with RCE at a dose of 1000 mg/kg died.
- The animals showed several clinical signs along the period post treatment: abnormal locomotion, hunched posture, piloerection, muscle relaxation and enlarged abdomen. These clinical signs observed are related with the general appearance and activity of the animal and do not affect the functional capacity. These signs could be attributed to the administration of the formulation at a high volume (20 mL/kg/animal). From the 4th day post administration, no clinical signs were observed in the animals administered at the dose of 1000 mg/kg.
- The evolution of the corporal weight of all the animals was normal.
  White areas were observed in the hepatic surface of all the male rats and of four female rats. The fifth female rat did not show macroscopic alterations. One of the male rats presented dilated ileum. The white areas observed on the hepatic surface in some rats are considered as remains of the product due to that the administration route was intraperitoneal.

CONCLUSION

- According to the results obtained, the Minimum Lethal Dose of RCE is greater than 1000 mg/kg when administered intraperitoneally in rats Sprague Dawley.

XIII.3. OTHER ANIMAL STUDIES WITH THE ROOSTER COMBS EXTRACT

XIII.3.1. STUDY OF THE EFFECTS OF THE ROOSTER COMBS EXTRACT ON HYALURONIC ACID CONCENTRATION IN A HORSE MODEL

This double-blind randomized controlled and prospective clinical pilot trial, was performed at the "*Universitat Autònoma"* of Barcelona, Spain, during 2005-06.

The study was published in 2009 (Carmona *et al,* 2009). See Annex VII for the complete study.

OBJECTIVE

To determine the effect of the oral administration of the RCE on safety and on clinical condition of horses with osteochondrosis (OCD).

EXPERIMENTAL DESIGN

12 horses aged between 8 and 36 months with a radiographic diagnose of OCD were randomly divided in two groups and assigned to receive orally 250 mg of the RCE or placebo during 60 days.

At the end of the treatment (day 60) and 30 days after finalization (day 90) a complete assessment of safety and clinical parameters were done.

RESULTS

Safety

- No adverse events related to the study products were reported.
- No significant changes were observed in plasma and synovial fluid analysis.

Efficacy

The results suggest that oral RCE administration increase HA concentration, which could be well related to improvements of the clinical condition of the affected joint.

XIII.4. HUMAN STUDIES

Human studies have also been performed on the RCE. Basically these are efficacy studies but with some evaluated safety parameters.

The safety of the RCE has been assessed in human studies. In two different studies (one in healthy adults and another in adults with osteoarthritis of the knee) no adverse effects have been reported as a result of the intake of the product at the recommended dose (80mg/d) during the studies periods.

XIII.4.1. ORAL ADMINISTRATION OF A YOGURT SUPPLEMENTED WITH A ROOSTER COMBS EXTRACT IN HEALTHY ADULTS

This randomised double-blind placebo-controlled nutrition intervention trial, evaluating the efficacy and safety of the RCE was performed in the Instituto POAL de Reumatología (Barcelona, Spain) between 2007 and 2008.

This study is actually under publication revision. See Annex VII for the complete study.

OBJECTIVES

- To determine the safety of the product supplemented with the RCE taking into consideration the adverse reactions, physic and vital constants examination and general tolerance.
- To determine the effect derived from the daily consumption of a supplemented yogurt with a RCE on the joint function.

EXPERIMENTAL DESIGN

The study enrolled 40 adults, 2 groups of 20 persons. All subjects were given one yogurt daily. Depending on the treatment assigned, the yogurts were supplemented with 80 mg of the RCE or, the yogurts were not supplemented. Total duration of this study for each person was of 12 weeks. All subjects carried out four visits along the study.

Safety parameters

Hereafter, some of the efficacy and safety parameters used in this study:

1. Body weight changes
2. Blood pressure
3. Heart rhythm
4. Subjective adverse effects
5. Physical adverse effects

RESULTS

- No adverse events were reported during the study.
- No significant changes were observed in body weight, blood pressure and heart rhythm after eating non-supplemented or supplemented yoghurts
- Regarding efficacy of the product, which was the main objective of the study, oral supplementation with RCE improved joint mechanics and muscle function as determined through isokinetic testing, thus attenuating risk factors of OA.

XIII.4.2. EFFECT OF ROOSTER COMBS EXTRACT ON PAIN RELIEF AND QUALITY OF LIFE IN SUBJECTS WITH KNEE OSTEOARTHRITIS: A PILOT RANDOMIZED DOUBLE-BLIND PLACEBO-CONTROLLED TRIAL

This randomised double-blind placebo-controlled clinical trial, evaluating the efficacy and safety of the RCE compared to placebo, for the improvement of quality of life in adults with osteoarthritis of the knee, was performed at Miami Research Associates (Miami), US, in 2005.

The study was published in 2008 (Kalman *et al,* 2008). See Annex VII for the complete study.

OBJECTIVES

- To determine the safety and tolerability of the RCE through the study.
- To determine the comparative differences between the RCE and placebo in quality of life and pain relief over 8 weeks.

EXPERIMENTAL DESIGN

The study enrolled 20 adults (both genders), age 40 and over, with a clinical diagnosis of osteoarthritis of the knee(s), but otherwise in good general health. Patients were given one capsule daily of the RCE after breakfast, at a dose of 80 mg/d, identical in appearance to active product. The randomisation ratio of the RCE to placebo was 1:1.

Efficacy and safety measures

Tolerability and safety parameters were the incidence and severity of adverse events reported throughout the study as well as changes in blood pressure, heart rate, and laboratory tests including complete blood cell count and biochemical profile. Treatment compliance was also recorded. Non-compliance was defined as taking less than 80% of the prescribed course of the study product. Use of rescue medication (paracetamol 500 mg) during the study period was also checked.

The primary efficacy variable was the comparative difference between the active and the placebo arms in scores of the pain subscale of WOMAC and bodily pain of SF-36v2.

The use of an unpaired Student t test to compare changes over time between treatment groups is algebraically equivalent to using a two-way linear model (ANOVA).

Analytical Populations

The *Safety population* consists of all 20 randomised subjects, 11 in the RCE group, and 9 in the placebo group.

The *Efficacy population* consists of 16 subjects, 8 in the RCE group, and 8 in the placebo group.

MAIN RESULTS

1. Safety Analysis

- No serious adverse events were reported during this study.
- Three adverse events were observed during the study period. One subject in the RCE group complained of acute non-target knee pain, unrelated to the study product, and voluntarily dropped out of the study. The two adverse events among placebo subjects, one diarrhea episode and one hypoesthesia of the tongue, were of mild intensity and were judged by the investigator as probably not related (diarrhea) and possibly related (hypoesthesia) to the study product. No significant changes were observed in vital signs, body weight, and results of laboratory tests.
- Overall, the RCE appeared to be well tolerated and safe within the study parameters (body weight or vital signs, and safety lab results).
- No alterations on biochemical profile or blood cell count were detected as a result of RCE supplementation.

2. Efficacy Analysis

Daily supplementation with RCE, 80 mg/day for 8 weeks, appeared to be effective in subjects with knee osteoarthritis for decreasing pain, improving physical function, and enhancing several aspects of quality of life.

XIII.5. TOXICITY OF SODIUM HYALURONATE

Published literature on toxicological studies shows that sodium hyaluronate is reasonably expected to be safe.

- Orl-Rat LD_{50} : > 800 mg/kg

  Yakuri to Chiryo. Pharmacology and Therapeutics, 12, 5369, 1984

- Ipr-Rat LD_{50} : 1770 mg/kg

  Yakuri to Chiryo. Pharmacology and Therapeutics, 12, 5369, 1984

- Scu-Rat LD_{50} : > 4 g/kg

  Yakuri to Chiryo. Pharmacology and Therapeutics, 12, 5369, 1984

- Orl-Mus LD_{50} : 2400 mg/kg

  Drugs in Japan (Ethical Drugs), 849, 1990

- Scu-Mus LD_{50} : 4 g/kg

  Yakuri to Chiryo. Pharmacology and Therapeutics, 12, 5369, 1984

- Scu-Dog LD_{50} : > 50 mg/kg

  Yakuri to Chiryo. Pharmacology and Therapeutics, 19 (suppl 1), S13, 1991

- Orl-Rbt LD_{50} : > 1 g/kg

  Drugs in Japan (Ethical Drugs), 849, 1990

- Ipr-Rbt LD_{50} : 1820 mg/kg

  Yakuri to Chiryo. Pharmacology and Therapeutics, 12, 5369, 1984

- Scu-Rbt LD_{50} : > 2 g/kg

  Yakuri to Chiryo. Pharmacology and Therapeutics, 12, 5369, 1984

Next abstract confirms the safety of sodium hyaluronate administered during 90 days in a subchronic toxicity study in rats. This study has special relevance due to that the sodium hyaluronate used was also obtained from an animal origin source (chicken) as our RCE.

- **Acute and subchronic oral toxicity in rats:**

Schauss AG, *et al*. Acute and subchronic oral toxicity studies in rats of a hydrolyzed chicken sternal cartilage preparation. *Food Chem. Toxicol*, 45(2):315-24, 2007:

Two acute and subchronic oral toxicity studies were conducted in rats to evaluate safety of a patented preparation of hydrolyzed chicken sternal cartilage (BioCell Collagen II) containing collagen type II, chondroitin sulfate, and hyaluronic acid. In the acute oral toxicity study, five males and five females of Sprague-Dawley rats were administered a single dose of 5000 mg of the test product per kg body weight and observed for 14 days. All animals survived and exhibited normal body weight gain throughout the study. Macroscopic necropsy examination conducted on day 15 revealed no gross pathological lesions in any of the animals. In the subchronic study, Sprague-Dawley rats (40 males, 40 females) were divided into four same-sex groups (10 animals/group). Animals in each group were administered daily either 0, 30, 300 or 1000 mg of the test product per kg of body weight for over 90 days. All animals survived and showed no significant changes in their body weights and histopathology. Although some differences were observed between the treated and control animals in several parameters, they were generally not dose-related or considered to be of toxicological significance. In conclusion, the results from the two oral toxicity studies with male and female young adult rats indicated that the test preparation from hydrolyzed chicken sternal cartilage collagen (BioCell Collagen II) was well tolerated at all four doses tested.

4. REFERENCES

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D. Martinez-Puig, I. Möller, C. Fernández, C. Chetrit (2009) *Efficacy of Oral Administration of Yoghurt Supplemented with a Natural Extract Containing Hyaluronic Acid (Mobilee™) in Adults with Mild Joint Discomfort: A Randomized, Double-blind, Placebo-controlled Intervention Study*. Joint Bone Spine *(under revision*).

D. Martinez-Puig, J.U. Carmona, D. Arguelles, R. Deulofeu, A. Ubia, M. Prades (2007) *Oral Hyaluronic acid administration improves osteochondrosis clinical symptoms and slightly increases intra-articular concentration of hyaluronic acid in a horse model: a pilot survey*. Osteoarthritis and Cartilage, Volume 15, Supplement 3, C62-C63.

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ANNEX I

Analysis Results Reports

ANNEX II

Analytical methods

Analytical procedures for the RCE

SODIUM HYALURONATE

Purpose

To determine the content in glucuronic acid in the product by acid hydrolysis of glycosaminoglycans and reaction of glucuronic acids with carbazole, to give a red coloured solution, colorimetrically quantifiable.

Reference method

Eur. Ph. Monograph 1472.

Reagents

- *Solution A*: Dissolve 19.08 g of sodium tetraborate decahydrate in approximately 200 ml of sulfuric acid and dilute to 250 ml with sulfuric acid. Keep in a cool place for not more than 2 months.
- *Solution B*: 0.125 % carbazole in absolute ethanol. Keep in a cool place for not more than 3 months.
- *Reference stock solution*: weigh accurately 140 mg of glucuronic acid, dissolve and dilute to 250 ml with distilled water.
- *Reference solutions*: prepare dilutions of the reference stock solution of 100, 50, 25 and 10 mg/l, diluting 20, 10, 5 and 2 ml of the stock solution to 100 ml with distilled water. Keep in a cool place for not more than 1 month.
- *Sample solution*: Weigh, accurately, about 170 mg (W) of the sample in a 100 ml volumetric flask. Dissolve and dilute to 100 ml with distilled water. Take 10 ml of this solution and dilute to 200 ml with distilled water in a volumetric flask.

Sample preparation and procedure

Add to 1 ml of sample solution in a screw capped test tube, cooled in an ice bath, 5 ml of solution A, mix and heat in a water bath at 95 - 100 ºC for 25 min. Cool in an ice bath and add 0.20 ml of carbazole solution (solution B). Shake vigorously and heat again to 95 - 100ºC for 25 min. Cool to room temperature and measure the absorbance (A) of the final solution at 520 nm, against a blank solution prepared the same way using 1 ml of distilled water instead of the sample solution. Perform the test in triplicate.

Notes:
The sample and the standard substance should be dried at 105ºC for at least 2 hours before weighing.
The absorbance of the blank against the borax solution (solution A) should not be more than 0.025.

Working scheme

Composition in ml of the solutions to be analyzed:

	BLANK	PROBLEM (X 3)	STANDARDS (X 4)
Sample solution	-------	1	-----
Standard solution	-------	-------	1
Distilled water	1	-------	-----
Solution A	5	5	5
Heat in a water bath at 95-100ºC for 25 minutes			
Cool in an ice bath			
Solution B	0.20	0.20	0.20
Heat in a water bath at 95-100ºC for 25 minutes			
Cool to room temperature in an ice bath			
Measure the absorbance at 520 nm			

Calculations

- Calibration function: Plot the standard solutions absorbance, A_{St}, against its concentration, C_{St} (mg/l).
- Calibration function type : A = - 0.015 + 0.0125 C ; r = 0.9999. From the calibration function and absorbance of the product, C_p is calculated in mg/l. The assay is expressed as content in Sodium Hyaluronate:

% Sodium Hyaluronate = Cp x 200 / W x 0.483

where : W is the sample weight in mg

: Cp is the concentration of the product

: 0.483 is the conversion factor from D-glucuronic acid to Sodium Hyaluronate

POTENTIOMETRIC DETERMINATION OF pH

Reference method

Eur. Ph. (*2.2.3*)

Equipment

- Crison pHmeter model 2001 (or equivalent), resolution: 0.01 pH unit, equipped with a glass-Ag/AgCl combined electrode, standardized with phosphate (pH : 7.00) and citrate (pH : 4.00) buffers.
- Temperature probe connected to the equipment.
- Alternatively use pH-meter Metrohm 691, with temperature probe. Other pH-meters provided with a temperature compensation probe can be used.

Procedure

Weigh 0.5 g of the sample and dilute to 50 ml with distilled water. Read the pH with the pH-meter, recently standardized using the temperature compensation probe. Keep the sample stirring while measuring.

MOHR METHOD (CHLORIDES)

Reference method

Mohr method

Reagents

- 5 % Potassium chromate in water
- 0.1 N Silver nitrate previously standardized with sodium chloride

Procedure

Dissolve about 0.5 g of the sample in 50 ml of distilled water. Check the pH is between 6.5 – 9.0 (otherwise adjust with acetic acid or sodium acetate, diluted to 10 %). Add 1 ml of 5 % potassium chromate solution and titrate with 0.1 N silver nitrate until an orange color appears. Follow the same procedure for the blank.

Calculations

$$\% \, Chlorides = \frac{\text{silver nitrate volume (ml)x 0.1 x 35.5}}{1{,}000 \; x \; sample \; weight \; (g)} x \; 100$$

DETERMINATION OF NITROGEN BY SULPHURIC ACID DIGESTION

Reference method

Eur. Ph. (*2.5.9*)

Reagents

- 0.1 N Sulphuric acid
- 96 % Sulphuric acid
- Kjeldahl catalyst (Cu-Se, 5 g pellet)
- Boric acid (4 % in water)
- Sodium hydroxide (30 % w/v)

Procedure

Weigh accurately 0.500 g of sample in a Kjedahl tube and add 20 ml of 96 % sulphuric acid and two pellets of catalyst Cu-Se. Mix gently and place the tube in the digestor.

Heat to about 375ºC. Once the liquid is transparent with a greenish colour (about 30 - 60 min), allow the tube to cool to ambient temperature. Add distilled water to buffer the posterior exothermic reaction when adding sodium hydroxide.

Set the method 324 in the distillator-titrator apparatus and introduce the weight value of the sample. Place the tube in the apparatus, which adds automatically the sodium hydroxide and the boric acid. The ammonia produced forms an ammonium salt with boric acid, which is then titrated with 0.1 N sulphuric acid. Once finished, the results are printed.

Parameters:

Distillator	Distilled water	10 ml
	Sodium hydroxide	75 ml
	Boric acid	60 ml
	Delay	0’05”
	Dist	4’30”
	Vapor	100 %
	Aspir	All

Calculations

$$\% \, Nitrogen = \frac{\text{Volume Sulfuric Acid x } 0.14}{Weight}$$

Crude protein determination :

For the protein determination, Nitrogen Kjedahl method is also applied to determine the percentage of total nitrogen in a specific sample.

Once the determination of the total nitrogen is concluded, the following formula is applied to determine the total crude protein:

Crude Protein = Nitrogen x 6.25 (conversion factor)

LOSS ON DRYING

Reference method

Eur. Ph. (*2.2.32*)

Procedure

Weigh 0.500 g in a weighing bottle previously dried under the same conditions prescribed for the substance to be examined. Dry the sample in an oven over diphosphorus pentoxide at 105ºC for 6 hours (until constant mass).

HEAVY METALS

Reference method

USP ⟨231⟩

Solutions

Standard Preparation

Into a 50-ml color-comparison tube pipet 2 ml of Standard Lead Solution (20 μg of Pb), and dilute with water to 25 ml. Adjust with 1 N acetic acid or 6 N ammonium hydroxide to a pH between 3.0 and 4.0, using short-range pH indicator paper as external indicator, dilute with water to 40 ml, and mix.

Test Preparation

Use a quantity, in g, of the substance to be tested as calculated by the formula 2.0 / (1000 L), in which L is the Heavy Metals limit, in percentage. Transfer the weighed quantity of the substance to a suitable crucible, add sufficient sulfuric acid to wet the substance, and carefully ignite at a low temperature until thoroughly charred (the crucible may be loosely covered with a suitable lid during the charring). Add to the carbonized mass 2 ml of nitric acid and 5 drops of sulfuric acid, and heat cautiously until white fumes no longer are evolved. Ignite, preferably in a muffle furnace, at 500 to 600ºC, until carbon is completely burned off. Cool, add 4 ml of 6 N hydrochloric acid, cover, digest on a steam bath for 15 minutes, uncover, and slowly evaporate on a steam bath to dryness. Moisten the residue with 1 drop of hydrochloric acid, add 10 ml of hot water, and digest for 2 minutes. Add 6 N ammonium hydroxide dropwise, until the solution is just alkaline to litmus paper, dilute with water to 25 ml, and adjust with 1 N acetic acid to a pH between 3.0 to 4.0, using short-range pH indicator paper as external indicator. Filter if necessary, rinse the crucible and the filter with 10 L of water, combine the filtrate and rinsing in a 50-ml color-comparison tube, dilute with water to 40 ml, and mix.

Procedure

To each of the tubes containing the *Standard Preparation* and the *Test Preparation*, add 2 ml of pH 3.5 acetate buffer, then add 1.2 ml of thioacetamide-glycerin base TS, dilute with water to 50 ml, mix, allow to stand for 2 minutes, and view downward over a white surface: the color of the solution from the *Test Preparation* is not darker than that of the solution from the *Standard Preparation*.

INDUCTIVELY COUPLED PLASMA-MASS SPECTROMETRY (ICP-MS)

Reference method

Eur. Ph. (*2.2.58*)

Equipment

- Sample-introduction system, consisting of a peristaltic pump delivering the solution at constant flow rate into a nebulizer
- Radio-frequency (RF) generator
- Plasma torch
- Interface region including cones to transport ions to the ion optics
- Mass spectrometer
- Detector
- Data-acquisition unit

Procedure

Sample preparations and sample introduction: The sample preparation usually involves a step of digestion of the matrix by a suitable method, for example in a microwave oven.

Furthermore, it is important to ensure that the analyte concentration falls within the working range of the instrument through dilution or preconcentration, and that the sample-containing solution can be nebulised in a reproductible manner.

Several sample-introduction systems tolerate high acid concentrations, but the use of sulphuric and phosphoric acids can contribute to background emission. Therefore, nitric and hydrochloric acids are preferable. The availability of hydrofluoric acid-resistant (for example perfluoroalkoxy polymer) sample-introduction systems and torches also allows the use of hydrofluoric acid. In selecting a sample-introduction method, the requirements for sensitivity, stability, speed, sample size, corrosion resistance and resistance to clogging have to be considered. The use of a torch is suitable for most requirements. The peristaltic pumps usually deliver the standard and sample solutions at a rate of 200-1000 µl/min.

In the case of organic solvents being used, the introduction of oxygen must be considered to avoid organic layers.

Choice of operating conditions: The standard operating conditions prescribed by the manufacturer are to be followed. Usually, different sets of operating conditions are used for aqueous solutions and for organic solvents. Suitable operating parameters are to be properly chosen:

- Selection of cones (material of sampler and skimmer)
- Support-gas flow rates (outer, intermediate and inner tubes of the torch)
- RF power
- Pump speed
- Selection of one or more isotopes of the element to be measured (mass)

Isotope selection: Isotope selection is made using several criteria. The most abundant isotope for a given element is selected to obtain maximum sensitivity. Furthermore, an isotope with the least interference from other species in the sample matrix and from the support gas should be selected. Information about isobaric interferences and interferences from polyatomic ions of carious types, for example hydrides, oxides, chlorides, etc..., is usually available in the software of ICP-MS instrument manufacturers.

MICROBIOLOGICAL EXAMINATION OF NON-STERIL PRODUCTS (TOTAL VIABLE AEROBIC COUNT)

Determine the total aerobic viable count by the plate-count method.

Reference method

Eur. Ph. (*2.6.12*)

Culture media

- CASO agar (TSA)
- Sabouraud Chloramphenicol agar (SCA)

Procedure

Preparation of the sample

Dissolve or dilute 10 g or 10 ml of the product to be examined in buffered sodium chloride-peptone solution pH 7.0 or another suitable liquid shown not to have antimicrobial activity in the conditions of the test, and adjust the volume to 100 ml with the same liquid. The characteristics of some products may need the use of larger volumes. If necessary, adjust to about pH 7.

Plate-count (for bacteria)

Using Petri dishes 9 to 10 cm in diameter, add to each dish a mixture of 1 ml of the prepared product and about 15 ml of liquefied agar medium (TSA) at not more than 45ºC. Alternatively, spread the prepared product on the surface of the solidified medium in a Petri dish of the same diameter. If necessary, dilute the prepared product so that a colony count of not more than 300 may be expected. Prepare at least two such Petri dishes using the same dilution and incubate at 30 - 35ºC for 5 days, unless a more reliable count is obtained in a shorter time. Count the number of colonies that develop. Calculate the results using plates with the greatest number of colonies but regarding 300 colonies per plate as the maximum consistent with good evaluation.

Plate-count (for fungi)

Using Petri dishes 9 to 10 cm in diameter, add to each dish a mixture of 1 ml of the prepared product and about 15 ml of liquefied agar medium (SCA) at not more than 45ºC. Alternatively, spread the prepared product on the surface of the solidified medium in a Petri dish of the same diameter. If necessary, dilute the prepared product so that a colony count of not more than 100 may be expected. Prepare at least two such plates using the same dilution and incubate at 20 - 25 ºC for 5 days, unless a more reliable count is obtained in a shorter time. Count the colonies that develop. Calculate the results using plates with not more than 100 colonies.

Interpretation of results

The total aerobic viable count is the sum of the bacteria count and the fungal count. If there is evidence that the same micro-organism may grow in both types of agar, the results must be corrected.

MICROBIOLOGICAL EXAMINATION OF NON-STERILE PRODUCTS (TEST FOR SPECIFIED MICRO-ORGANISMS)

Identification of *Escherichia coli*, *Salmonella, Pseudomonas aeruginosa* and Staphylococcus aureus. These four micro-organisms indicate the potential simultaneous presence of other pathogen micro-organisms which are ecologically related.

Reference method

Eur. Ph. (2.6.13)

Culture media

- Baird-Parker agar (BP) (*Staphylococcus* selective agar base acc. Baird-Parker)
- Brillant-green phenol-red lactose sucrose agar (BPLS Agar (USP))
- CASO agar (TSA)
- Tryptic soy broth (TSB) (CASOY broth)
- Pseudomonas selective agar base (cetri) (Cetrimide agar)
- Lactose broth (C LAC)
- Leifson agar (DC) (Deoxycholate citrate agar acc. to Leifson, modified)
- MacConkey agar (MCA)
- MacConkey broth (MCB)
- Pseudomonas agar F base for detection of Fluorescein (PAF)
- Pseudomonas agar P base for detection of Pyocyanin (PAP)
- Tetrathionate brillant-green bile enrichment broth (TBG broth modified)
- Triple sugar iron agar (TSI agar)
- Xylose lysine desoxycholate agar (XLD agar)

Procedure

- Preparation of the sample for *E. coli* and *Salmonella* identification
- Weigh, next to a free flame, 10 ± 0.1 g of sample into a sterile 100 ml bottle, blazing the bottles and spatules used (in case the sample must be less than 10 g, add sufficient peptonated water to obtain a 10 % solution w/v). Add 90 ml of lactose broth (C LAC). Once the sample is completely dissolved, homogenise and incubate at 35 - 37ºC for 18 - 24 h. Homogenise the liquid by agitation (homogenate A).
- Preparation of the sample for *P. aeruginosa* and *S. aureus* identification
- Weigh, next to a free flame, 10 ± 0.1 g of sample into a sterile 100 ml bottle, blazing the bottles and spatules used. Add 90 ml of sterile peptonated water (peptone-sodium chloride buffer solution pH 7.0). Shake until sample is completely dissolved (in case the sample must be less than 10 g, add sufficient peptonated water to obtain a 10 % solution w/v).

Determination of Escherichia coli

Dissolve 10 g of the product to be examined in 90 ml buffered sodium chloride-peptone solution pH 7.0. Use 10 ml to inoculate 100 ml of TSB, homogenise and incubate at 35–37ºC for 18–48 h. Shake the container, transfer 1 ml to 100 ml of MCB and incubate at 43-45ºC for 18-24h. Subculture on plates of agar MCA at 35-37ºC for 18-72 h. Growth of red, non-mucoid colonies of gram-negative rods indicates the possible presence of E. coli. This is confirmed by suitable biochemical test. The product passes the test if such colonies are not seen or if the confirmatory biochemical test are negative.

Determination of Salmonella

Dissolve 10 g of the product to be examined in TSB, homogenise and incubate at 35–37ºC for 18–24 h. Transfer 1 ml of the enrichment culture to 10 ml of TBG and incubate at 41-43ºC for 18-24h. Subculture on at least 2 different agar media chosen from agar DC, XLD and BPLS. Incubate at 35-37ºC for 18-72 h. The probable presence of salmonella is indicated by the growth of cultures having the following appearance:

- agar DC: well-developed, colourless colonies,
- agar XLD: well-developed, red colonies, with or without black centers,

- agar BPLS: small, transparent colourless or pink or opaque-white colonies, often surrounded by a pink or red zone.

Transfer separately a few of the suspect colonies to agar TSI in tubes, using surface and deep inoculation. The presence of salmonellae is provisionally confirmed if in the deep inoculation but not in the surface culture there is a change of colour from red to yellow and usually a formation if gas, with or without production of hydrogen sulphide in the agar. Precise confirmation may be carried out by appropriated biochemical and serological test. The product passes the test if colonies of the type described do not appear or if the confirmatory biochemical and serological test are negative.

Determination of Pseudomonas aeruginosa

Dissolve 10 g of the product to be examined in TSB, homogenise and incubate at 35–37ºC for 18–24 h. Shake the container and subculture on plate of agar medium Ctrimide.

If growth of colonies of gram-negative rods, usually with a greenish fluorescence, occurs, subculture the suspect colonies on Pseudomonas Agar Medium for Detection of Fluorescin (PAF), to detect fluorescin, and on Pseudomonas Agar Medium for Detection of Pyocyanin (PAP), to detect pyocyanin. Incubate at 30 - 35 ºC for 24 - 72 h. The probable presence of Pseudomonas aeruginosa is indicated by the growth of cultures having the following appearance:

- PAF fluorescein: Colourless to yellow gram-negative rods. Yellowish fluorescence at 366 nm with yellow to green pigment. Oxidase test positive.
- PAP pyocyanin: Greenish gram-negative rods. Blue to greenish pigment. Oxidase test positive.

The product passes test if:

- there is no growth in the TSB medium
- there is no growth of colonies of gram-negative rods, usually with a greenish fluorescence, on plates of Cetrimide agar
- the confirmatory biochemical tests are negative
- oxidase test
- fluorescin and/or pyocyanin test
- RapID NF Plus (AES laboratoires ref. IDS 5005), API 20 NE or equivalent.

Determination of *Staphylococcus aureus*

Dissolve 10 g of the product to be examined in 90 ml buffered sodium chloride-peptone solution pH 7.0. Use 10 ml to inoculate 100 ml of TSB, homogenise and incubate at 35–37ºC for 18–48 h. Subculture on plates of agar BP and incubate at 35-37ºC for 18-72 h. Black colonies of gram-positive cocci, surrounded by a clear zone indicate the presence of S. aureus. Confirmation may be effected by suitable biochemical test. The product passes the test if colonies of the type described do not appear on agar BP or if the confirmatory biochemical test are negative.

ANNEX III

Quality certificate of rooster combs

ANNEX IV

Food grade and not genetically modified enzyme certificates

ANNEX V

Rooster combs recipes

EVIDENCE OF USE OF CHICKEN COMBS AS INGREDIENT IN RECIPES

Source: http://www.designobserver.com/archives/entry.html?id=26733

COCK-A-DOODLE-DON'T

Sautéed Rooster Comb. (Day 1) 2007.

The American writer Calvin Trillin used to tell a story about one of his daughters who, upon being invited one day to accompany her father to a new restaurant, inquired meekly if it would be all right if she "took a bagel just in case." This tale was repeated to me frequently as a child, as this was the role I played in my family: I was the worrier, the apprehensive and non-adventurous one, the child always wary of novelty — especially if it meant eating something weird.

As it happened, I married the opposite personality type — someone who, on a recent trip to Spain, did what he always does when we travel: he went native. And then he ordered rooster comb.

Sautéed Rooster Comb. (Day 5) 2007.

Rooster comb is a Catalan delicacy, and has been compared to chicken feet: it's flaccid and tan-colored, with diaphanous skin and a wobbly texture. Poke it with your fork, and it has the consistency of fatty flesh: it's rubbery, like the glove the evil penguin wore on his head in The Wrong Trousers. Bizarre, but then again, eating is often a springboard for such associations, which helps to recast the whole idea of food as entertainment. (Which in turn leads to things like Iron Chefs and extreme cakes) Like breathing and sleeping, we eat to live. It's hard to imagine a television program about the dramatic highs and lows of oxygen intake, but food is something else altogether.

Where food is concerned, the relationship between what things look like and how we respond exists at its most primal level: what is a gut reaction, after all, if not something that attacks your gut? Food preferences are personal, idiosyncratic, and odd. They're also framed by things like appetites, religious preferences and allergies; swayed by things seasonal, products regional, and palettes likely to be unpredictably mercurial. And no matter what it is or how picky we are (or aren't) — the fact remains that what food looks like has a huge bearing on what we taste.

Personally, where anthropomorphic meets edible is where I draw the line.

And while there was perhaps something eerily beautiful about that floating, disenfranchised fan of rooster muscle — beautiful, that is, in the most abstract way possible — I couldn't get past its unsettling resemblance to Foghorn Leghorn. (I am sure that the sandwich spread known as

Goober's is just as horrifying to most people, but it doesn't look like a body part.) The fact that this curious delectation was ordered as a side dish for a meal consisting of foie gras and octopus is enough to make even the most intrepid epicure reconsider the virtues of a vegan diet.

Of course, the discussion and visual documentation of weird food is something well-represented by a host of websites maintained by foodies and the people who love them: from 1970s Weight Watcher's Recipe Cards (thank you, Bethany Johns) to Musk-Flavored Life Savers, here's a sure-fire way to drop those extra pounds. Then again, who am I to disparage rooster as a menu item? Alaina Browne praises a New York restaurant that offers up this dish "braised to gummy brownness," while critic Andrew Zimmern describes them here as a "gelatinous delight."

Sautéed Rooster Comb. (Day 15) 2007.

This summer has dawned with a host of food-related films, from Ratatouille to No Reservations the pink dougnut of an "O' in The Simpson's Movie logo, reminding us that audiences don't only like to eat food, they like to look at it, too: in cookbooks and on television, in magazines and in retail establishments, in markets and on restaurant tables all over the planet. Which, in the global world of anything-goes culinary inclusivism, just happens to include rooster comb. Mercifully for the less adverturesome among us, it also includes bagels.

Source: http://www.rivistaidea.it/ideatourism/engl/finanziera.html

PIEDMONTESE STYLE MEAT DISH (ITALIAN CUISINE)

Ingredients for 6 people:
150 g. chicken liver; 150 g. chicken crests; 150 g. calf's spinal marrow; 150 g. calf's sweetbreads; 150 g. veal rump; 2 pickled gherkins; 40 g. dried Boletus mushrooms; 70 g. of butter; 2 dl. Sweet white wine; 1 cup of white flour; salt; pepper.

Preparation: Soak the mushrooms for 20 minutes. Place the sweetbread in a saucepan, cover with water and bring to the boil for 5 minutes, then remove them and cool them under running water; drain remove the film and chop them up. Wash the spinal marrow and slice it. Take another saucepan and parboil the chicken crests for 5 minutes, sprinkle with salt and rub with the hands so to make plucking easier. Immerse in cold water to remove the salt. Then clean the chicken livers and chop, cut the veal rump into pieces. Place the butter in a saucepan and melt, add the veal rump and fry for a few minutes stirring with a wooden spoon. Coat the sweetbread, spinal marrow and crests with flour and then also place them in the frying pan. Allow to cook for 5 minutes and then add the chicken liver, also coated in flour, and after a further 5 minutes add the wine; season with salt and freshly ground pepper, add the drained and chopped mushrooms and the roughly chopped gherkins. Allow to cook over a very low heat covering the pan with a cover for a further 15 minutes, ensure that it is sufficiently seasoned and serve hot.

Source: From the May 17, 2004 issue of New York Magazine.
http://newyorkmetro.com/nymetro/food/features/n_10380/index2.html

"EXTREME EATING"

Cock's combs, "a gelatinous sponge of meat." (Photo credit: James Wojcik)

The chefs who are writing weird food onto fine-dining menus, though, are chasing more than just thrills.

Andy Nusser, of Casa Mono, first spotted cockscombs in Barcelona and knew he had to serve them. It's tempting to suspect that they are there for the sake of pure novelty, to lure the army of restless New Yorkers who crave the exotic if only to consume it and transform it into the banal as quickly as possible.

But for chefs, says Nusser, it is the buzz of a new ingredient, the same jonesing for new sensation that hooked them on the business in the first place. A cockscomb is, Nusser says, "a gelatinous sponge of meat that will take on any flavor you will add to it, and that's exciting." Like many chefs, he faces the challenge of making new foods and flavors accessible without obscuring the ingredient itself. In this case, that means taking advantage of the braised combs' flavor-sponginess to infuse them with comforting hints of hearty pot roast and a plummy, hoisin sweetness. But the combs still violate a cardinal rule of mainstream American cuisine: that a food not resemble the animal it came from. And there is no mistaking when the dish arrives that you are eating a plate full of rooster-head tops.

Source: http://italianfood.about.com/library/rec/nr1084.htm

UMIDO DI RIGAGLIE DI POLLO COL SEDANO -- STEWED CHICKEN GIBLETS WITH CELERY

This is from Pellegrino Artusi's *Scienza In Cucina e L'Arte di Mangiar Bene,* and was omitted from my translation (the Art of Eating Well, Random House) for want of space. Though some might now make a face at something made from chicken organs, it's a very traditional dish of a kind that is now enjoying a revival in elegant Tuscan restaurants.

"If the necks and feet of the chickens are added to this dish, it becomes the family meal we all know," Says Artusi. "However, when preparing it for a more solemn occasion, it should be made with just the livers, crests, unborn eggs, gonads, and gizzards (only if the latter have been blanched in broth and trimmed of all soft muck).

"Begin by cooking two or three sticks of celery, cut into one-inch lengths, till one third done in salted water. Then mince a few slices of prosciutto and a little onion, sauté the mixture in butter, and, when the onion's lightly browned, add the gizzards, cut into thirds, then a pinch of corn starch, then the chicken livers, cut into halves, and the remaining ingredients. Season the mixture with salt, pepper, and a inch of mixed spices, and after a few minutes sprinkle it with broth and a couple tablespoons of tomato sauce. Meanwhile, sauté the celery in butter in a separate pan, and when it is cooked, stir it into the giblets. Simmer the stew until the meat is tender, adding more broth if need be, and serve, in a sformato di riso."

Source: Platina's On Right Pleasure and Good Health**,** published by The University of North Carolina at Asheville. 200 pages, 6" x 9"

ON CHICKEN ROLL (Chicken crests, livers, and testicles… here starts the really strange organ meats.)

Divide crests of chickens in three pieces, livers in four, and leave testicles whole. Cut lard into bits, but do not pound. Cut up finely two or three ounces of veal fat, or, instead of fat, add beef or calves marrow. Use as much as will be enough of ginger, cinnamon, and sugar. Mix all these with about forty dried sour cherries; then put in a roll made suitable for it from finely ground meal. It can be cooked in an oven or under cover on the hearth. When it is half-cooked, put over it two beaten egg yolks and a bit of saffron and verjuice.

Source: Wine Spectator Magazine June 30, 2003
http://www.winespectator.com/Wine/Archives/Show_Article/0,1275,4160,00.html

TASTING THE FUTURE BY THOMAS MATTHEWS

Sergi Arola spent six years cooking with Adrià at El Bulli; now he's chef at La Broche in Madrid. The restaurant is a minimalist white cube; the wine list an adventurous tour of Spain's alta expresión pioneers. Arola uses earthy, rustic ingredients, such as rooster combs and sardines, but transforms them with imaginative garnishes. A gratin of cuttlefish, for example, is served with a scoop of ice cream flavored with olive oil and garlic, which melts into the hot sauce. The flavors make perfect sense; the clash of temperatures takes the dish to a new dimension.

Source : http://www.canalacademie.com/Un-petit-metier-de-bouche.html

UN PETIT MÉTIER DE BOUCHE : FABRICANT DE CRÊTES DE COQ !

Histoire et Gastronomie, la chronique du Dr Jean Vitaux

Pour un parfait vol au vent ou une délicate bouchée à la reine, prenez des crêtes de coq ! Et faute d'en trouver, un certain monsieur Lecoq, bienfaiteur de l'humanité, n'a pas hésité à en fabriquer. L'histoire mérite d'être contée. Un petit métier de bouche comme il en existait au XIX^{e} siècle.

Le XIX^{e} siècle a été le siècle du vol au vent et des bouchées à la reine.
La formule du vol-au-vent a été détaillée par Antonin Carême, et la bouchée à la reine aurait été inventée pour Marie Leszczynska, reine de France, épouse de Louis XV. La farce traditionnelle de ces délicats feuilletages était faite de ris de veaux (ou d'agneaux dits béatilles), de petites quenelles et de blancs de volaille, d'abats (rognons et testicules de volailles), et de crêtes de coq, nappés de sauce financière ou suprême.

La mode était telle que les crêtes de coq étaient très recherchées. Privat d'Anglemont dans *Paris anecdote*, préfacé par le grand gastronome et poète Charles Monselet, nous a laissé l'extraordinaire histoire de Monsieur Lecoq, ce bienfaiteur de l'humanité, qui fabriquait les crêtes de coq.
Le père Lecoq habitait dans une cour du faubourg Saint Antoine où il existait une machine à vapeur reliée à un arbre de telle sorte que chaque locataire pouvait y adapter une machine. Monsieur Lecoq, constatant le manque de crête de coq, et leurs imperfections naturelles, décida d'y remédier. **Il prit des palais de veau, de bœuf ou de mouton qu'il faisait longuement bouillir, puis qu'il passait sous le balancier de la machine pour créer de fausses crêtes de coq à l'emporte-pièce.** Si les crêtes de coq ainsi réalisées étaient plus régulières que les crêtes naturelles des coqs, elle ne présentaient qu'un seul défaut : elles n'avaient des tubercules (ou papilles) que d'un seul côté, contrairement aux vraies crêtes de coq.
Ce bienfaiteur de l'humanité, comme il se qualifiait, estimait que chaque matin, il entrait à Paris vingt cinq mille à trente mille poulets, répartis entre les tables bourgeoises et les restaurateurs, pâtissiers et rôtisseurs. Il n'en restait plus encore que dix à douze mille crêtes de coq disponibles pour les vols au vent, timbales, coquilles et autres préparations de cet aliment alors si recherché, peut être en raison des propriétés aphrodisiaques qu'on leur prêtait. Mr Lecoq estimait donc qu'il

rendait service en fabriquant la quantité de crêtes de coq nécessaire. Les vendant 15 centimes la douzaine aux restaurateurs et 20 centimes aux cuisinières bourgeoises, il fit fortune.

Source: **http://www.lhotellerie-restauration.fr/lhotellerie/Recettes/Chefs/Ducasse/Gnocchi-cretes-et-rognons.htm**

GNOCCHI, CRÊTES ET ROGNONS DE COQ

La Riviera, Alain Ducasse, Albin Michel

Ouvrage disponible auprès de Editions BPI

Ingrédients

- 4 crêtes de coq
- 4 rognons de coq
- 2 homards de 400 g chacun
- 50 g de girolles
- 50 g de morilles
- 4 lamelles de truffe
- 40 g de parmesan râpé
- 2 dl de fond blanc
- 1,5 dl de jus de veau
- 1 dl de jus de truffe
- 100 g de beurre
- 3 cuillères à soupe d'huile d'olive
- 12 brins de cerfeuil
- 4 cuillères à soupe de jus de citron
- 1 cuillère à soupe de farine
- Sel
- Poivre

Pour les gnocchi :

- 250 g de ricotta
- 25 g de farine de blé blanche
- 1 oeuf
- 1 dl d'huile d'olive
- 4 pincées de noix de muscade râpée

- Sel
- Poivre

Recette

- Versez 1 litre 1/2 d'eau dans une casserole. Salez-la. Ajoutez-y la farine, en fouettant et 3 cuillères à soupe de jus de citron. Portez à ébullition. Plongez les crêtes de coq dans ce "blanc". Laissez-les cuire 2 heures à petits frémissements.
- Plongez les homards dans de l'eau bouillante salée. Faites-les cuire 7 minutes. Rafraîchissez-les dans de l'eau glacée, puis décortiquez-les. Coupez quatre médaillons dans les queues et réservez les pinces entières.
- Coupez le bout terreux du pied des champignons. Lavez-les. Faites-les cuire séparément : les girolles dans une noix de beurre et 1/2 cuillerée à soupe d'huile, bien dorées. Les morilles juste chauffées dans une noix de beurre, mouillées de 2 cuillères à soupe de fond blanc et étuvées 15 minutes.

Préparez les gnocchi :

- Mettez tous les ingrédients dans une terrine. Mélangez le tout au fouet, vivement, jusqu'à ce que vous obteniez une pâte lisse et homogène. Faites chauffer de l'eau dans une marmite de 26 cm. Salez-la. Lorsqu'elle frémit, faites tomber dans l'eau des petites quenelles de ricotta formées entre deux cuillères à café : les gnocchi. Lorsqu'ils remontent à la surface, ils sont cuits. Plongez-les aussitôt dans de l'eau glacée, puis égouttez-les et réservez-les sur une plaque huilée.
- Plongez les rognons dans de l'eau bouillante salée. Laissez-les cuire 2 minutes puis plongez-les dans de l'eau glacée. Lorsque les crêtes sont cuites, parez-les et partagez-les en trois.
- Faites fondre 50 g de beurre dans une sauteuse de 24 cm. Mettez les crêtes, les champignons, le jus de truffe et les lamelles de truffe dans la sauteuse. Faites chauffer, puis ajoutez le jus de veau. Laissez mijoter 3 minutes. Faites fondre le beurre restant dans une poêle de 26 cm. Faites chauffer les gnocchi dans le beurre chaud et faites-les légèrement blondir, puis poudrez-les de parmesan en les retournant délicatement dans le beurre.

- Dans une petite poêle, faites chauffer l'huile d'olive et faites-y sauter les médaillons et les pinces de homard, à feu vif.
- Egouttez les rognons : coupez-les en deux verticalement et ajoutez-les dans la sauteuse, avec 1 cuillère à soupe de jus de citron. Mélangez délicatement.
- Répartissez le contenu de la sauteuse dans les assiettes (crêtes, champignons, truffes et rognons) et glissez entre ces ingrédients les médaillons de homard. Parsemez de gnocchi. Posez une pince de homard sur le tout. Nappez du jus contenu dans la sauteuse, réchauffé. Décorez de cerfeuil et servez aussitôt.

Source: http://scope.lefigaro.fr/restaurants/restauration/gastronomique/l-r214025--alain-ducasse-au-plaza-athenee/static/

ALAIN DUCASSE AU PLAZA ATHÉNÉE

Genre : Gastronomique
Cuisine : Française
Spécialité : Viandes, Poissons - Fruits de mer

Au célèbre Plaza Athénée, dans le non moins célèbre restaurant auquel il a donné son nom (et ses trois étoiles...), c'est Alain Ducasse qui conçoit et Christophe Moret, son émérite chef cuisinier, qui exécute une cuisine traditionnelle et moderne à la fois où toutes les régions de France sont représentées. Au menu de la « carte printemps » : pâtes mi-séchées aux ris de veau, crêtes et rognons de coq; volaille de Bresse rôtie, pommes boulangère et morilles à peine crémées; bar de ligne agrumes/poivres, ventrèche caramélisée...

Les plus : Terrasse, Air conditionné, Salons privés, Cave à vins, Cheminée, Voiturier

Source : http://chefsimon.com/crete.htm

LES FICHES TECHNIQUES DU CHEF SIMON

COMMENT PREPARER LES CRÊTES DE COQ

Bannies de cuisines, délaissées par les cuisiniers, oubliées ...

Les crêtes de coq pourtant encore citées dans les manuels historiques ayant encore cours aujourd'hui...

On remplace avec facilité les vraies crêtes de coq par de la langue écarlate

maladroitement taillée pour donner le change.
Pourtant la crête de coq peut aussi se travailler.
Entourée d'un mystère technique quasi-mystique, où il faut être initié pour maîtriser l'occulte, il n'y a rien de plus enfantin que de préparer ces excroissances qui servent en grande cuisine classique comme élément de garniture.Rien d'extraordinaire sur le plan gustatif, seulement un clin d'oeil au passé.. ce passé où
rien ne se perdait !!

Amusez vous donc, pour le plaisir d'épater .. mais de vous épater vous-même !

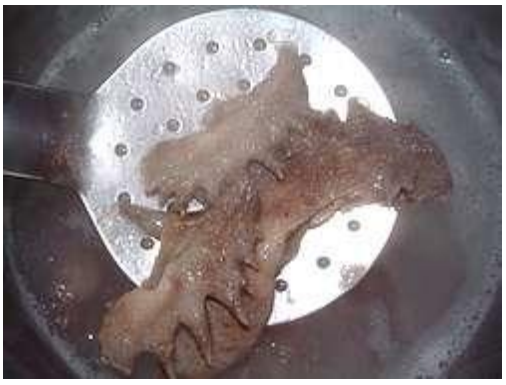
On commence par dégorger les crêtes dans l'eau froide
On les aura piquées à l'aiguille pour les plus grosses afin de les débarrasser de leur sang résiduel

BLANCHIMENT	
	
Placer les crêtes dans une casserole	Couvrir d'eau froide et porter à petite ébullition une dizaine de minutes
	
Égoutter et rafraîchir sous l'eau froide.	Replacer dans la casserole rincée pour cuire dans un **blanc simplifié**

"BLANC" POUR CRETES DE COQ

Eau, farine, acide citrique ou jus de citron et gros sel.

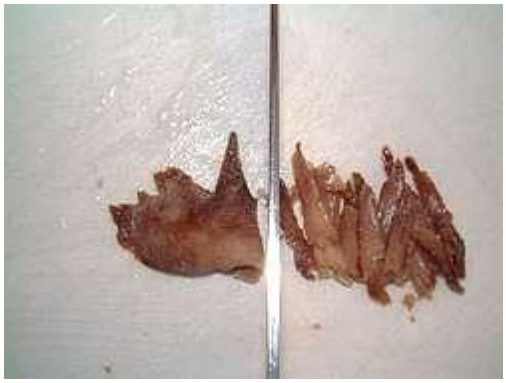
Porter à petite ébullition et plonger les crêtes une dizaine de minutes à petite ébullition.



Une cuiller de farine Une demie d'acide citrique (ou à défaut une tranche de citron)	Cuire une vingtaine de minutes à petite ébullition
	
On pique pour s'assurer de la cuisson.	La crête est tendre
	
On grattera sommairement la surface fragile de la crête.	La texture est gélatineuse

	
La surface à nettoyer est insidieusement solidaire de la partie à conserver (La partie gélatineuse)	Après le grattage sommaire, rincer sous l'eau.
	
On terminera par un frottage au sel fin, entre le pouce et l'index, avec soin et calmement.	Très vite l'action abrasive du sel élimine les parties sèches de al crête

On voit apparaître la crête débarrassée de sa surface presque tubéreuse.



Il ne reste alors qu'à détailler selon l'emploi attendu

Ici en Julienne pour une garniture de timbale de volaille

Etonnant et suprenant. Pathétique car n'apportant rien de véritablement révolutionnaire dans le plat lui même hormis une texture Cela fait partie de notre patrimoine culinaire à conserver au rang des curiosités culturelles !

Source: http://gourmetymerlin.blogspot.com/2009/04/tagliolini-con-langostinos-y-crestas-de.html

TAGLIOLINI CON LANGOSTINOS Y CRESTAS DE GALLO

Una receta sencillita pero resultona que le debo a la enorme mejora en la selección de productos que está experimentando Orvi, una pequeña tienda de mi pueblo en la que últimamente, además de embutidos de calidad se puede encontrar una creciente selección de quesos (por ejemplo el Scamorza ahumado que compré ayer), algas, variedad de pastas italianas, conservas de gama alta (desde las clásicas de pescado a tomates secos o crestas de gallo) y una pequeña selección de vinos interesantes que, en mi opinión, tienen su punto débil en la conservación en tienda pero que, mejorando ese aspecto, son, sin duda, la mejor oferta de la comarca.

Así que, tras una visita de viernes tarde a esta tienda, cosa que se está convirtiendo en una costumbre, ayer preparamos para cenar estos.

TAGLIOLINI AL HUEVO CON LANGOSTINOS Y CRESTAS DE GALLO CONFITADAS

Por un lado se cuece la pasta, mientras que por otra parte se saltean con un diente de ajo picado los langostinos y las crestas de gallo, que lavé bien ya que venían confitadas en grasa de pato. Se añade sal y, en el último momento, añadí unas hojas picadas de albahaca fresca.

Por otro lado piqué un poco que lechuga hoja de roble, para compensar un poco el posible exceso de grasa que pudiese aportar el confitado de las crestas, y preparé un aliño de aceite de oliva, limón, pimienta negra y sal. Con servir el salteado sobre la pasta recién cocina hubiese sido suficiente, pero añadir la lechuga y el aliño le da al plato un toque más de ensalada tibia que tampoco está mal.

El resultado es un sabroso mar y montaña, dominado por el sabor de los langostinos, el aroma de la albahaca y la textura inconfundible de las crestas (similar a la de los callos, para quien no las conozca). Muy interesante.

Source: http://www.elmundo.es/metropoli/2006/03/08/restaurantes/1141836469.html

READ'S. Mallorca. Ctra. vieja Santa María-alaró (Santa María) | 971 14 02 61 | No cierra| 70 euros | www.readshotel.com

El Read's es un restaurante situado en una típica mansión mallorquina del siglo XVI, reconstruida y reconvertida en un hotel de lujo con 26 habitaciones. En el año 2003 ganó el premio al Mejor y Excelente Hotel Rural de Europa Condé Nast Johansens. También en el mismo año se le concedió una estrella en la guía Michelin. La cocina de Marc Fosh es audaz y comprometida, pero nunca transgresora. Un ejemplo son los filetes de gallo San Pedro y crestas de gallo con aceite de naranja, anís verde y crema ligera de vino tinto. Por Íñigo Morales de Rada.

Source: **http://entrecolycollechuga.blogspot.com/search/label/crestas%20de%20gallo**

ENSALADA TIBIA DE CRESTAS DE GALLO

INGREDIENTES:

patatas
pimentón dulce
crestas de gallo confitadas en grasa de pato (las venden en lata)
aceite de oliva virgen extra

PROCEDIMIENTO:

Ya hicimos una tapa de parecida manera ahora variamos la forma de presentación, la presentamos como una ensalada tibia, las patatas y las crestas deben estar mínimamente tibias.
Estas crestas que usamos las venden en latas ya confitadas en su grasa de pato, en tiendas de productos gourmets, ya me entendéis, es que no quiero hacer publicidad *" de gratis"* que dicen los catalanes.
Recuerdo que en tiempos pretéritos, cuando yo llevaba pantalones cortos, o sea la semana pasada, cuando en casa se mataba un pollo, un pollo de los de reglamento, de aquellos de 9 meses que se habían criado picoteando por el corral, la cresta era el apéndice más solicitado de aquel apetitoso guiso que había preparado mi madre y que nos lo comíamos convencidos de que en aquella casa se comía mejor que los domingos en el cielo. Mira por donde ahora sigue

siendo un bocado delicado, ya se que a los muy impresionables les dará un poco de corte, pero atrévete son deliciosas...

Para empezar cocemos unas patatas en agua hirviendo, enteras, con piel incluida. Cuando estén cocidas (depende del tamaño, pero si son grandes, unos 20 minutos) las sacamos y reservamos sin cortar para que se mantengan calientes a la hora de emplatar.

Por otro lado colocamos al baño maría la lata de las ínclitas crestas, están bañadas en grasa de pato y poco a poco se licuará.

Para servir cortamos la patata en rodajas, aliñamos con un poco de la grasa licuada de las crestas y espolvoreamos por encima el pimentón dulce, sólo nos quedará poner los apéndices de los gallos por encima.

Yo que tu probaría este plato...

ANNEX VI

Allergenicity report

ANNEX VII

Toxicity studies performed with the rooster combs extract

ANNEX VIII

Anticipated intakes report

ANNEX IX

Commercialized food supplements containing sodium hyaluronate

Hereafter are described some oral supplements commercialized in the USA containing hyaluronic acid.

From Schiff webpage
www.schiffvitamins.com

Schiff®

Improves Popular Move Free® Joint Supplement Formula With Addition of Joint Fluid

SALT LAKE CITY, November 30, 2004 – Schiff®, one of America's most trusted and respected natural supplement brands, has improved its best-selling Move Free® joint care supplement line by adding Joint Fluid. Joint Fluid (Hyaluronic Acid) is a compound that helps joints glide easily and smoothly.

Joint Fluid is Hyaluronic Acid (HA). Synovial fluid is made up of molecules of Hyaluronic Acid that enable cartilage to glide easily and quietly over other parts of the joint. Joint Fluid (HA) molecules also assist in nutrient delivery to joint tissues. Orally ingested HA has been shown to be absorbed and concentrated in joints.

"Based on considerable research and study of the oral delivery and absorption of HA commissioned by our company and published in The FASEB Journal, we are pleased to introduce the addition of Joint Fluid to our Move Free® formula. This product improvement further ensures that our consumers are receiving comprehensive, hard working joint care products," said Dr. Luke Bucci, Ph.D., Vice President of Research for Schiff®.

The 1,500 milligrams glucosamine/1,200 milligrams chondroitin blend in Move Free® products is designed to support joint cartilage and maintain healthy joint function.** Glucosamine, a natural amino sugar produced by the body, plays an important role in providing the building blocks for healthy cartilage.** Chondroitin, a naturally occurring nutrient in the connective tissue, attracts water to the area, and it makes the cartilage more elastic and better able to absorb shock.**

The new Move Free® products will be available beginning in December at club, grocery, mass merchandiser and drug stores nationwide.

From Purity Products web site:
www.purityproducts.com/

Vital-HA Max Formula

"Supports Healthy Skin, Joints & Vision*"

- **Supports Healthy Skin, Joints & Vision**
- **90 softgel capsules, 30 day supply**
- **Special Super Saver Price: $29.95**

Product Description

Vital H.A. Max is the NEXT generation hyaluronic acid product featuring a combination of 150 mg. H.A. (from 1500 mg BioCell Clollagen II per serving) along with health supporting Omega 3 oils, Folic acid and more. Also features other breakthrough nutrients such as alpha lipoic acid and ferulic acid. It's a unique Healthy Aging product and contains superior quality Hyaluronic Acid (H.A.) in a high performance 10% extract... to support healthy joints, skin, collagen levels and more.* Please check out our brand new H.A. Skin Rejuvenating Serum, which is getting great reviews.

From Kinetic Technologies webpage
http://www.kinetictech.net/ksupplements.htm

KINETIC JOINT III

KINETIC JOINT III offers a unique patent pending formulation combining hyaluronic acid (HA), glucosamine and chondroitin. HA works synergistically with glucosamine and chondroitin to help keep your joints healthy.

In general, hyaluronic acid has been proven to enhance cartilage-producing cells, increase synthesis of HA and improve joint flexibility. Adult serving is three capsules per day. 60 and 180 capsule per bottle.

From Kinetic Technologies webpage
http://www.kinetictech.net/ksupplements.htm

HA 30

A new concept in human health!

HA 30 is an oral supplement containing hyaluronic acid (HA). HA is well documented in maintaining joint health. HA is also important in vision, integrity of tissues, elasticity of skin, as a circulatory lubricant and several other physiological functions. Unfortunately as we age, our natural levels of HA decrease. With HA 30 you can supplement your diet with this important substance. Until recently, to receive HA you had to receive an injection. Now it is available in oral form. Adult serving is 1-2 capsules per day. Available in 60 and 180 capsule bottles.

From Purity Products webpage
http://www.purityproducts.com/ultimate-ha.asp?

Ultimate HA Formula (Hyaluronic Acid)
"Supports Healthy Skin, Joints & Vision*"

Product Description

Ultimate HA Formula (Hyaluronic Acid) is a breakthrough healthy aging formula used to support joint health, skin health and more. Purity's Ultimate HA contains 150 mg of Hyaluronic Acid (HA) per serving, in a high performance 10% extract... made from patented BioCell Collagen II and blended with chondroitin sulfate.* Hyaluronic Acid is a key component of collagen which makes

up the material between cells and provides structure to various organs like the eyes, joints and skin. Check out our brand new HA Skin Rejuvenating Serum, which is getting great reviews.

From Natures Health products webpage
http://www.coral1.com/syn.html

SYNTHOVIAL 7

Synthovial 7™ is an oral solution made from premium grade Hyaluronic acid with a molecular weight between 2.4 -- 2.8 million Daltons. Hyaluronic acid (HA) is a polymer and it can come in varying molecular weights. Studies have shown more benefits with the higher molecular weight HA polymers. More cushioning and lubricating properties for the joints and tissues, more moisture retaining qualities for the skin. We have the highest molecular weight oral HA in the market.

Synthovial7™ is a liquid solution of hyaluronic acid. Oral liquid solutions by nature are absorbed by the body easier and quicker than tablets and capsules. Enjoy the benefits faster --- joint pain relief, clearer, smoother, younger looking skin and all the other anti-aging benefits of Hyaluronic acid all throughout the body.

Seven drops in a glass of water once a day is all it takes. You don't have to worry if you have taken the third of four doses of those capsules or tablets today. You don't have to make doctor appointments for those costly and painful injections. You don't have to take extra medications to combat the harmful side effects of your prescription. And no more of those smelly and messy creams.

Synthovial 7™ is Hyaluronic acid that comes from an extra cellular substance produced by a bacteria in a laboratory. There are no animal derivatives. Since HA is native to the human body and our product is not derived from animal tissue, hypersensitivity is not a concern. There are no known side effects.

IT WORKS!!!

Hyaluronic acid (HA) has been proven by numerous medical studies to alleviate pain and suffering from arthritis of the knee and other joints. Synthovial 7™ is an oral solution made from premium grade hyaluronic acid with a molecular weight between 2.4 - 3.0 million Daltons.

Hyaluronic acid is a polymer and it can come in varying molecular weights. Studies have shown more benefits with the higher molecular weight HA polymers. More cushioning and lubricating properties for the joints and tissues, more moisture retaining qualities for the skin.

IT WORKS FAST!!!

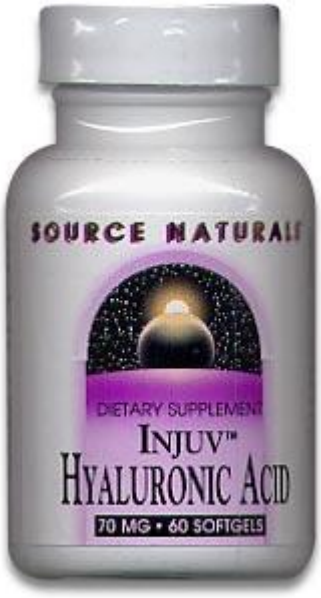
Synthovial 7™ is a liquid solution of hyaluronic acid. Oral solutions by nature are absorbed by the body easier and quicker than tablets and capsules. Enjoy the benefits faster - joint pain relief, clearer, smoother, younger looking skin and all the other anti-aging benefits of hyaluronic acid all throughout the body.

IT IS EASY TO TAKE!!!

A full dropper, one (1) ml. to a glass of water once a day is all it takes. You don't have to worry if you have taken the third dose of your medication today. You don't have to make doctor appointments for those costly and painful injections. You don't have to take extra medications to combat the harmful side effects of your prescription. And no more of those smelly and messy creams.

IT IS SAFE!!!

Synthovial 7™ is hyaluronic acid that comes from an extra cellular substance produced by a bacteria. There are no animal derivatives. Since HA is native to the human body and our product is not derived from animal tissue, hypersensitivity is not a concern. There are no known side effects.



From Source Naturals webpage
http://www.sourcenaturals.com/products/GP1671.html

Hyaluronic Acid Injuv™

Injuv™ is a revolutionary new form of hyaluronic acid. Hyaluronic acid plays an integral role in maintaining and regulating moisture within the tissues and facillitates the transport of nutrients into the cells and the transport of metabolic waste. It is found in all the body's tissues, with

the highest concentrations located in the extracellular matrix of the skin and in the synovial fluid that bathes the joints and cartilage. Optimum levels of hyaluronic acid are essential for the health of joints and cartilage. Injuv™ provides the only hyaluronic acid that has undergone a natural enzyme cleaving technique for greater absorption.

Supplement Facts Serving Size: 2 Softgels	
	Amount %DV
Injuv™	140 mg †
Yielding 9% Hyaluronic Acid	12.6 mg †

Other Ingredients: rice bran oil, gelatin (capsule), glycerin, water, beeswax, and titanium dioxide. Warning: If you are pregnant or breastfeeding, consult your physician before using this product.

Suggested Use: 2 softgels twice daily with a meal for the first 3 weeks. Dosage can then be decreased to two softgels daily.

SKU	Count/Type	Suggested Retail
SN1549	30 sg	$ 0.00
SN1550	60 sg	$ 0.00

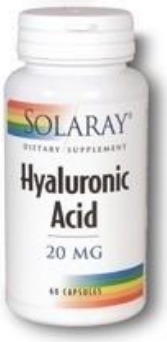
From Source Naturals webpage
http://www.sourcenaturals.com/products/GP1671.html

Skin Eternal™ Hyaluronic Acid

Supports Skin Fitness

Hyaluronic acid and collagen are vital components of skin structure that decline as we age. They are responsible for the skin's moisture, suppleness, and elasticity. Patented BioCell Collagen II™ is made from 100% pure cartilage, which has undergone an absorption enhancing hyrdolyzation process that yields low molecular weight hyaluronic acid, chondroitin sulfate, and Collagen Type II peptides.

These elements found in BioCell Collagen II™ make it a multifaceted ingredient which may help support healthy skin function and appearance, as well as help support joint comfort and function.



From Nutraceutical webpage
http://www.nutraceutical.com/about/solaray.cfm

HYALURONIC ACID

Description:	Solaray® Hyaluronic Acid is enteric coated, which helps the capsules survive the stomach acid and pass into the small intestine where it is absorbed. This formula contains Ascorbic Acid to further assist metabolism of Hyaluronic Acid.
Item#:	92402
Size:	60ct 20mg
Suggested Retail Price:	$30.19
Directions:	As a dietary supplement, take 1 capsule one to two times daily with a meal or glass of water.
Serving Size:	1 Capsule 60 Servings per container

Ingredients:	**Amount per serving:**	**% Daily Value: +**
Hyaluronic Acid (Microbial Fermentation)	20 mg	~
Vitamin C (as Ascorbic Acid)	50 mg	83%

Key to Ingredients:
~ = Daily Value not established.
+ = Percent Daily Value is based on a 2,000 calorie diet.
† = Values differ depending on age
‡ = Values differ depending on age

Other Ingredients:	Cellulose, Gelatin Capsule, Glycerol Triacetat, Silica and Magnesium Stearate
Other:	See product label for more information.

From Lane Labs webpage
http://www.lanelabs.com/products.asp?productID=TOKIcolor

TOKI

When the Japanese began exporting their exotic porcelain vases in the seventeenth century, the world was suddenly exposed to purity never before seen. Translucence and a smoothness of texture that remains unsurpassed. Soft-to-the-touch yet firm in shape. Finely symbolic, it strikes a clear note of wealth and health, and also graces the surface of dolls and china.

TOKI reintroduces a youthful look and feel to skin with an extraordinary ingredient base that includes the moisture-rich retention of Hyaluronic and Dermantanic acids. These acids replenish skin from within, and combine with Collagen Peptide and Vitamin C to restore a more youthful quality. When modified with HAI Amino Acid Extract from Hijiki seaweed for optimal absorption, the appearance of fine lines and wrinkles are dramatically reduced. Skin's texture becomes smoother, tighter and more refined*. The porcelain returns.

Skin Nourishment From Within

Generations of Japanese women understand that skin is nourished primarily from within. Now the unique combination of Japanese wisdom and science has led to a patented natural complex able to replenish skin quality from within.

Noticeable results in 45 days or less

Rediscover youthful, healthy, radiant skin with TOKI, a supplement that reverses the effects of the environment by replenishing your skin from within. Your skin tone, moisture level and resiliency are significantly improved. At 45 days, skin texture is smoother and tighter and the appearance of the appearance of fine lines and wrinkles is diminished.

Aging Skin

Aging skin appears as finely lined, wrinkled, dry or rough, discolored and patchy. On the surface, the problem appears to be the outer layer, but aging skin actually occurs in the dermis, or second layer of skin where collagen, elastin and moisture reside. When certain components are diminished and compromised, skin becomes dehydrated. The porcelain cracks. TOKI works from the inside out to replenish depleted nutrients your skin and bones need

Rejuvenated Skin

TOKI™ reintroduces a youthful look and feel to skin with an extraordinary ingredient base that includes the moisture-rich retention of Hyaluronic and Dermantanic acids. These acids replenish skin from within, and combine with Collagen Peptide and Vitamin C to restore a more youthful quality. When modified with HAI Amino Acid Extract from Hijiki seaweed for optimal absorption, the appearance of fine lines and wrinkles are dramatically reduced. Skin's texture becomes smoother, tighter and more refined. The porcelain returns.

From Natural Health Consultants webpage
http://www.naturalhealthconsult.com/Monographs/SynovoDerma.html

SYNOVODERMA

by Allergy Research Group

WHAT IS IT?

SynovoDerma contains Hyaluronic Acid powder (9% min. Hyaluronic acid) 210 mg per 3 sofgel capsules. Other inert ingredients are rice bran oil, yellow beeswax, and titanium dioxide.

WHAT DOES IT DO?

SynovoDerma promotes skin health. A unique formulation includes a natural active ingredient called Hyaluronic Acid (HA). HA is essential to maintaining fluid balance within cells and is depleted in great amounts as we age. This precious nutrient can now be enhanced through oral supplementation of HA with a lower molecular weight to increase absorption of this unique formulation. HA is extracted from the cockscomb. While most HA products work externally (on a superficial level) SynovoDerma works internally, providing moisture to the inner layers of the epidermis.

HA is involved in the rejuvenation of skin, joint tissue, and immune function.

CAUTIONS

No side-effects are known so far.

See Disclaimer.

DOSE

Take 6 softgels daily for 20 days, then reduce dosage to 3 softgels daily, or as directed by a healthcare practitioner. May be taken with or without food.

From Lucy's Barkery webpage
http://www.lucysbarkery.com/glyco-oral.htm

GLYCO-ORAL

Glyco Oral Hyluronic Acid is easy to use and affordable. Just use for our five drops two times a day on food or treats.

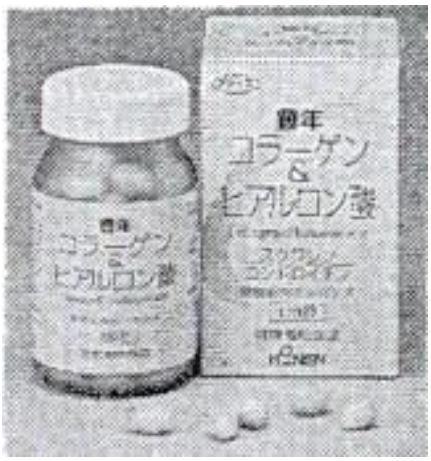
1 OZ BTL CONTAINS:

Extra Strength Formula

New Premium Grade of our special formula Hyaluronic Acid for oral use.

Honen Supplement - Collagen and Hyaluronic Acid Capsules

Report Number: 187163
Manufacturer: Honen Corp.
Country: Japan
Industry: Health & Beauty Aids
Category Number: 363
Category Term: Vitamins & Supplements
Ingredients: Collagen; Hyaluronic Acid; Silk Peptides; Squalene; Chondroitin; Hatomugi (Adlay) Oil
Package Type: Bottle; Box
Shelving: General
Innovation: Not Innovative
Description: Honen Corp.: Honen Collagen and Hyaluronic Acid. A healthy and beauty nutritional supplement, collagen is a fibrillar protein (extracted from animal skin) important for skin care, hyaluronic acid is a mucopolysaccharide which helps to retain moisture in the skin (often used in cosmetics). This supplement also contains silk peptides rich in amino acids, squalene, chondroitin and hatomugi [adlay] oil. Daily dose 4-6 capsules, 120 capsules Y3,800, 240 capsules Y5,800.
Total SKUs: 2
Publication Name: Japanscan
Publication Date: January 25, 2000



Oral preparations containing collagens, hyaluronate, and elastins to stimulate metabolism of skin cells.
Nakamura, Koretaka; Yamamori, Taiji. (Rainbow Co., Ltd., Japan). Jpn. Kokai Tokkyo Koho (2001), 9 pp. CODEN: JKXXAF JP 2001226286 A2 20010821 Patent written in Japanese. Application: JP 2000-37426 20000216. CAN 135:185463 AN 2001:603501 CAPLUS (Copyright 2002 ACS)

Abstract
This invention relates to oral compns. comprising type 1 collagen peptides, hyaluronic acid, and elastins to activate skin cells. The compns. further comprise vitamin C, placental exts., chondroitin sulfate, DNA/nucleic acids, Pueraria mirifica, papaya enzymes. The compns. can be in the forms of health food.

Antiaging and cosmetic effects of dietary hyaluronic acid (extracellular matrix extract).
Yamamoto, Hajime. Department of Research and Development, Adaptogen Pharmaceutical Co., Ltd., Japan. New Food Industry (1998), 40(11), 33-41. CODEN: NYFIAM ISSN: 0547-0277. Journal written in Japanese. CAN 130:261936 AN 1999:47337 CAPLUS (Copyright 2002 ACS)

Abstract
We all know that humans cannot live without water, and the decrease of the body fluid will cause senility. The extracellular matrix fluid keeps its viscosity by hydration of mucopolysaccharides and some proteins, but the most important and potent element to hold water is hyaluronic acid (HA). HA is widely distributed throughout the body, and the skin also contains plenty of HA so that decreasing HA in the skin with aging leads to skin dryness and wrinkles. Now digestible HA has been developed which can be taken with other everyday foods. We are sure that our HA was absorbed orally and created HA-rich skin which is moist and smooth.

ANNEX X

Viral Safety report