SCIENTIFIC OPINION

Scientific Opinion on the substantiation of health claims related to bonito protein peptide and maintenance of normal blood pressure (ID 1716) pursuant to Article 13(1) of Regulation (EC) No 1924/2006[1]

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

SUMMARY

Following a request from the European Commission, the Panel on Dietetic Products, Nutrition and Allergies was asked to provide a scientific opinion on a list of health claims pursuant to Article 13 of Regulation (EC) No 1924/2006. This opinion addresses the scientific substantiation of health claims in relation to bonito protein peptide and maintenance of normal blood pressure. The scientific substantiation is based on the information provided by the Member States in the consolidated list of Article 13 health claims and references that EFSA has received from Member States or directly from stakeholders.

The food constituent that is the subject of the health claims is bonito protein peptide. The Panel considers that bonito protein peptide (or LKPNM, amino acid sequence Leu-Lys-Pro-Asn-Met) is sufficiently characterised.

The claimed effect is "natural blood pressure support". The target population is assumed to be the general population. In the context of the proposed wording, the Panel assumes that the claimed effect refers to the maintenance of a normal blood pressure. The Panel considers that maintenance of normal blood pressure is a beneficial physiological effect.

In weighing the evidence, the Panel took into account that no human studies from which conclusions could be drawn for the scientific substantiation of the claim were provided, and that results from the animal and *in vitro* studies submitted are not sufficient to predict the occurrence of an effect of bonito protein peptide (LKPNM) on blood pressure in humans.

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[1] On request from the European Commission, Question No EFSA-Q-2008-2452, adopted on 09 July 2010.

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[3] Acknowledgement: The Panel wishes to thank for the preparatory work on this scientific opinion: The members of the Working Group on Claims: Carlo Agostoni, Jean-Louis Bresson, Susan Fairweather-Tait, Albert Flynn, Ines Golly, Marina Heinonen, Hannu Korhonen, Martinus Løvik, Ambroise Martin, Hildegard Przyrembel, Seppo Salminen, Yolanda Sanz, Sean (J.J.) Strain, Inge Tetens, Hendrik van Loveren and Hans Verhagen. The members of the Claims Sub-Working Group on Cardiovascular Health/Oxidative Stress: Antti Aro, Marianne Geleijnse, Marina Heinonen, Ambroise Martin, Wilhelm Stahl and Henk van den Berg.

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On the basis of the data presented, the Panel concludes that a cause and effect relationship has not been established between the consumption of bonito protein peptide (or LKPNM, amino acid sequence Leu-Lys-Pro-Asn-Met) and the maintenance of normal blood pressure.

KEY WORDS

Bonito protein peptide, fish, blood pressure, health claims.

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INFORMATION AS PROVIDED IN THE CONSOLIDATED LIST

The consolidated list of health claims pursuant to Article 13 of Regulation (EC) No 1924/2006[4] submitted by Member States contains main entry claims with corresponding conditions of use and literature for similar health claims. EFSA has screened all health claims contained in the original consolidated list of Article 13 health claims which was received by EFSA in 2008 using six criteria established by the NDA Panel to identify claims for which EFSA considered sufficient information had been provided for evaluation and those for which more information or clarification was needed before evaluation could be carried out[5]. The clarifications which were received by EFSA through the screening process have been included in the consolidated list. This additional information will serve as clarification to the originally provided information. The information provided in the consolidated list for the health claims which are the subject of this opinion is tabulated in Appendix C.

ASSESSMENT

1. Characterisation of the food/constituent (ID 1716)

The food constituent that is the subject of the health claim is bonito protein peptide.

Dried bonito (type of fish) is a traditional Japanese seasoning known as Katsuobusi. Bonito protein peptide is obtained after hydrolysis with thermolysin (or digestion) of bonito muscle. From the unknown number of peptides present in the digest, eight peptides have been isolated that could inhibit the angiotensin-converting enzyme (ACE) *in vitro*. The amino acid sequences of these ACE-inhibitory peptides are Ile-Lys-Pro-Leu-Asn-Tyr, Ile-Val-Gly-Arg-Pro-Arg-His-Gln-Gly, Ile-Trp-His-His-Thr, Ala-Leu-Pro-His-Ala, Phe-Gln-Pro, Leu-Lys-Pro-Asn-Met, Ile-Tyr, and Asp-Tyr-Gly-Leu-Tyr-Pro. Of these, Leu-Lys-Pro-Asn-Met (or LKPNM) is considered the main "active" ingredient (IC50 = 2.4 μmol/L). Thermolysin-digested dried bonito containing 0.17 % (w/w) of LKPNM, also known as "Katsuobushi Oligopeptide (KO)", is available as a food ingredient on the Japanese market (Yokoyama et al., 1992; Fujita et al., 2000).

The Panel considers that the food constituent, bonito protein peptide (or LKPNM, amino acid sequence Leu-Lys-Pro-Asn-Met), which is the subject of the health claim, is sufficiently characterised.

2. Relevance of the claimed effect to human health (ID 1716)

The claimed effect is "natural blood pressure support". The Panel assumes the target population is the general population.

In the context of the proposed wording, the Panel assumes that the claimed effect refers to the maintenance of normal blood pressure.

Blood pressure is the pressure (force per unit area) exerted by circulating blood on the walls of blood vessels. Elevated blood pressure, by convention above 140 mmHg (systolic) and/or 90 mmHg (diastolic), may compromise the normal arterial and cardiac function.

The Panel considers that maintenance of normal blood pressure is a beneficial physiological effect.

3. Scientific substantiation of the claimed effect (ID 1716)

Seven references were provided for the substantiation of this claim. Four of these provide data on the angiotensin-converting enzyme (ACE) inhibitory activity of bonito protein digest *in vitro* and/or antihypertensive effects of this digest in spontaneously hypertensive rats (SHR). The remaining three

[4] Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods. OJ L 404, 30.12.2006, p. 9–25.
[5] Briefing document for stakeholders on the evaluation of Article 13.1, 13.5 and 14 health claims: http://www.efsa.europa.eu/en/ndameetings/docs/nda100601-ax01.pdf

references reported on human intervention studies which assessed the effects of Katsuobushi Oligopeptide (KO)" standardised by its LKPNM content on blood pressure (Fujita et al., 1997a; 1997b; 2001).

A randomised crossover study was performed in 35 borderline and mildly hypertensive Japanese subjects with an average blood pressure level of 150/100 mmHg (Fujita et al., 1997a). No information on use of antihypertensive drugs was provided in the publication. During active intervention, subjects received 3 g per day of KO, yielding an intake of 5 mg per day of LKPNM, for a period of eight weeks. Half of the subjects (group 1) took the test food during the first 8-week period, and the other half (group 2) during the second 8-week period. During control periods, no placebo supplement or other type of intervention were provided. Blood pressure values in the last week of the test food period were compared to those of the control period in the 30 subjects who completed the study. No biological parameters of the renin-angiotensin system were assessed to check ACE-inhibition. The Panel notes that the sample size was small, that this study was neither blinded nor placebo controlled, that carry-over effects were not assessed, and that antihypertensive medication use in the study subjects was not reported. The Panel considers that no conclusions can be drawn from this reference for the scientific substantiation of the claimed effect.

The same investigators also conducted a 2x5-week randomised crossover study (Fujita et al., 1997b) in which 37 borderline and mildly hypertensive Japanese subjects with an average blood pressure of 151/100 mmHg consumed a KO-containing soup, followed by a placebo soup, or vice versa. No information on use of antihypertensive medications was provided. During active treatment, subjects had a daily intake of 3 g/d of KO, providing 5 mg/d of LKPNM. There were no drop-outs during the study. The Panel notes that no direct statistical comparisons were made between the intervention and the placebo periods, and that no information on use of antihypertensive medications by the study subjects was provided. The Panel considers that no conclusions can be drawn from this reference for the scientific substantiation of the claimed effect.

Fujita et al. (2001) obtained a "stronger KO" (S-type KO) by ultra-filtration that contained 0.34 % (w/w) of LKPNM and had a two-fold higher antihypertensive activity in spontaneously hypertensive rats after oral administration, compared with the original KO. The effect of 1.5 g per day of S-type KO (in tablets) on blood pressure was tested against placebo in a randomised, double-blind cross-over study in 65 borderline and mildly hypertensive Japanese subjects with an average blood pressure level of 149/93 mmHg. No information on use of antihypertensive medications was provided. Four subjects dropped out during the study. No biological parameters of the renin-angiotensin system were assessed to check ACE-inhibition. The Panel notes that no direct statistical comparisons were made between the intervention and the placebo periods, and that no information on the use of antihypertensive medications by the study subjects was provided. The Panel considers that no conclusions can be drawn form this reference for the scientific substantiation of the claimed effect.

Four references provided data on the ACE-inhibitory activity of bonito protein digest *in vitro* (Yokoyama et al., 1992; Fujita et al., 2000) and/or on the antihypertensive effects of this digest in spontaneously hypertensive rats (Fujita et al., 1995; Fujita and Yoshikawa, 1999). The Panel considers that while effects shown in animal and *in vitro* studies may be used as supportive evidence, human studies are required for the substantiation of a claim, and evidence provided in animal and *in vitro* studies alone is not sufficient to predict the occurrence of an effect of bonito protein peptide (LKPNM) on blood pressure in humans.

In weighing the evidence, the Panel took into account that no human studies from which conclusions could be drawn for the scientific substantiation of the claim were provided, and that results from the animal and *in vitro* studies submitted are not sufficient to predict the occurrence of an effect of bonito protein peptide (LKPNM) on blood pressure in humans.

The Panel concludes that a cause and effect relationship has not been established between the consumption of bonito protein peptide (or LKPNM, amino acid sequence Leu-Lys-Pro-Asn-Met) and maintenance of normal blood pressure.

CONCLUSIONS

On the basis of the data presented, the Panel concludes that:

- The food constituent, bonito protein peptide (or LKPNM, amino acid sequence Leu-Lys-Pro-Asn-Met), which is the subject of the health claim, is sufficiently characterised.
- The claimed effect is "natural blood pressure support". The target population is assumed to be the general population. Maintenance of normal blood pressure is a beneficial physiological effect.
- A cause and effect relationship has not been established between the consumption of bonito protein peptide (or LKPNM, amino acid sequence Leu-Lys-Pro-Asn-Met) and maintenance of normal blood pressure.

DOCUMENTATION PROVIDED TO EFSA

Health claims pursuant to Article 13 of Regulation (EC) No 1924/2006 (No: EFSA-Q-2008-2452). The scientific substantiation is based on the information provided by the Member States in the consolidated list of Article 13 health claims and references that EFSA has received from Member States or directly from stakeholders.

The full list of supporting references as provided to EFSA is available on: http://www.efsa.europa.eu/panels/nda/claims/article13.htm.

REFERENCES

Fujita H, Yokoyama K, Yasumoto R and Yoshikawa M, 1995. Antihypertensive effect of thermolysin digest of dried bonito in spontaneously hypertensive rat. Clinical and Experimental Pharmacology & Physiology, 22, 304-305.

Fujita H, Yasumoto R, Hasegawa M and Ohshima K, 1997a. Human volunteers study on antihypertensive effect of "katsuobushi oligopeptide" (I). Japanese Pharmacological Therapy, 25, 147-151.

Fujita H, Yasumoto R, Hasegawa M and Ohshima K, 1997b. Human volunteers study on antihypertensive effect of "katsuobushi oligopeptide (II). A placebo-controlled study on the effect of "peptide soup" on blood pressure in borderline and hypertensive subjects. Japanese Pharmacological Therapy, 25, 153-157.

Fujita H and Yoshikawa M, 1999. LKPNM: a prodrug-type ACE-inhibitory peptide derived from fish protein. Immunopharmacology, 44, 123-127.

Fujita H, Yokoyama K and Yoshikawa M, 2000. Classification and antihypertensive activity of angiotensin I-converting enzyme inhibitory peptides from food proteins. Journal of Food Science, 54, 564-569.

Fujita H, Yamagami T and Ohshima K, 2001. Effects of an ace-inhibitory agent, katsuobushi oligopeptide, in the spontaneously hypertensive rat and in borderline and mildly hypertensive subjects. Nutrition Research, 21, 1149-1158.

Yokoyama K, Chiba H and Yoshikawa M, 1992. Peptide inhibitors for angiotensin I-converting enzyme from thermolysin digest of dried bonito. Bioscience, Biotechnology and Biochemistry, 56, 1541-1545.

APPENDICES

APPENDIX A

BACKGROUND AND TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The Regulation 1924/2006 on nutrition and health claims made on foods[6] (hereinafter "the Regulation") entered into force on 19th January 2007.

Article 13 of the Regulation foresees that the Commission shall adopt a Community list of permitted health claims other than those referring to the reduction of disease risk and to children's development and health. This Community list shall be adopted through the Regulatory Committee procedure and following consultation of the European Food Safety Authority (EFSA).

Health claims are defined as "any claim that states, suggests or implies that a relationship exists between a food category, a food or one of its constituents and health".

In accordance with Article 13 (1) health claims other than those referring to the reduction of disease risk and to children's development and health are health claims describing or referring to:

a) the role of a nutrient or other substance in growth, development and the functions of the body; or

b) psychological and behavioural functions; or

c) without prejudice to Directive 96/8/EC, slimming or weight-control or a reduction in the sense of hunger or an increase in the sense of satiety or to the reduction of the available energy from the diet.

To be included in the Community list of permitted health claims, the claims shall be:

(i) based on generally accepted scientific evidence; and

(ii) well understood by the average consumer.

Member States provided the Commission with lists of claims as referred to in Article 13 (1) by 31 January 2008 accompanied by the conditions applying to them and by references to the relevant scientific justification. These lists have been consolidated into the list which forms the basis for the EFSA consultation in accordance with Article 13 (3).

ISSUES THAT NEED TO BE CONSIDERED

IMPORTANCE AND PERTINENCE OF THE FOOD[7]

Foods are commonly involved in many different functions[8] of the body, and for one single food many health claims may therefore be scientifically true. Therefore, the relative importance of food e.g. nutrients in relation to other nutrients for the expressed beneficial effect should be considered: for functions affected by a large number of dietary factors it should be considered whether a reference to a single food is scientifically pertinent.

It should also be considered if the information on the characteristics of the food contains aspects pertinent to the beneficial effect.

SUBSTANTIATION OF CLAIMS BY GENERALLY ACCEPTABLE SCIENTIFIC EVIDENCE

[6] OJ L12, 18/01/2007

[7] The term 'food' when used in this Terms of Reference refers to a food constituent, the food or the food category.

[8] The term 'function' when used in this Terms of Reference refers to health claims in Article 13(1)(a), (b) and (c).

Scientific substantiation is the main aspect to be taken into account to authorise health claims. Claims should be scientifically substantiated by taking into account the totality of the available scientific data, and by weighing the evidence, and shall demonstrate the extent to which:

(a) the claimed effect of the food is beneficial for human health,

(b) a cause and effect relationship is established between consumption of the food and the claimed effect in humans (such as: the strength, consistency, specificity, dose-response, and biological plausibility of the relationship),

(c) the quantity of the food and pattern of consumption required to obtain the claimed effect could reasonably be achieved as part of a balanced diet,

(d) the specific study group(s) in which the evidence was obtained is representative of the target population for which the claim is intended.

EFSA has mentioned in its scientific and technical guidance for the preparation and presentation of the application for authorisation of health claims consistent criteria for the potential sources of scientific data. Such sources may not be available for all health claims. Nevertheless it will be relevant and important that EFSA comments on the availability and quality of such data in order to allow the regulator to judge and make a risk management decision about the acceptability of health claims included in the submitted list.

The scientific evidence about the role of a food on a nutritional or physiological function is not enough to justify the claim. The beneficial effect of the dietary intake has also to be demonstrated. Moreover, the beneficial effect should be significant i.e. satisfactorily demonstrate to beneficially affect identified functions in the body in a way which is relevant to health. Although an appreciation of the beneficial effect in relation to the nutritional status of the European population may be of interest, the presence or absence of the actual need for a nutrient or other substance with nutritional or physiological effect for that population should not, however, condition such considerations.

Different types of effects can be claimed. Claims referring to the maintenance of a function may be distinct from claims referring to the improvement of a function. EFSA may wish to comment whether such different claims comply with the criteria laid down in the Regulation.

WORDING OF HEALTH CLAIMS

Scientific substantiation of health claims is the main aspect on which EFSA's opinion is requested. However, the wording of health claims should also be commented by EFSA in its opinion.

There is potentially a plethora of expressions that may be used to convey the relationship between the food and the function. This may be due to commercial practices, consumer perception and linguistic or cultural differences across the EU. Nevertheless, the wording used to make health claims should be truthful, clear, reliable and useful to the consumer in choosing a healthy diet.

In addition to fulfilling the general principles and conditions of the Regulation laid down in Article 3 and 5, Article 13(1)(a) stipulates that health claims shall describe or refer to "the role of a nutrient or other substance in growth, development and the functions of the body". Therefore, the requirement to describe or refer to the 'role' of a nutrient or substance in growth, development and the functions of the body should be carefully considered.

The specificity of the wording is very important. Health claims such as "Substance X supports the function of the joints" may not sufficiently do so, whereas a claim such as "Substance X helps maintain the flexibility of the joints" would. In the first example of a claim it is unclear which of the various functions of the joints is described or referred to contrary to the latter example which specifies this by using the word "flexibility".

The clarity of the wording is very important. The guiding principle should be that the description or reference to the role of the nutrient or other substance shall be clear and unambiguous and therefore be specified to the extent possible i.e. descriptive words/ terms which can have multiple meanings should be avoided. To this end, wordings like "strengthens your natural defences" or "contain antioxidants" should be considered as well as "may" or "might" as opposed to words like "contributes", "aids" or "helps".

In addition, for functions affected by a large number of dietary factors it should be considered whether wordings such as "indispensable", "necessary", "essential" and "important" reflects the strength of the scientific evidence.

Similar alternative wordings as mentioned above are used for claims relating to different relationships between the various foods and health. It is not the intention of the regulator to adopt a detailed and rigid list of claims where all possible wordings for the different claims are approved. Therefore, it is not required that EFSA comments on each individual wording for each claim unless the wording is strictly pertinent to a specific claim. It would be appreciated though that EFSA may consider and comment generally on such elements relating to wording to ensure the compliance with the criteria laid down in the Regulation.

In doing so the explanation provided for in recital 16 of the Regulation on the notion of the average consumer should be recalled. In addition, such assessment should take into account the particular perspective and/or knowledge in the target group of the claim, if such is indicated or implied.

TERMS OF REFERENCE

HEALTH CLAIMS OTHER THAN THOSE REFERRING TO THE REDUCTION OF DISEASE RISK AND TO CHILDREN'S DEVELOPMENT AND HEALTH

EFSA should in particular consider, and provide advice on the following aspects:

- Whether adequate information is provided on the characteristics of the food pertinent to the beneficial effect.
- Whether the beneficial effect of the food on the function is substantiated by generally accepted scientific evidence by taking into account the totality of the available scientific data, and by weighing the evidence. In this context EFSA is invited to comment on the nature and quality of the totality of the evidence provided according to consistent criteria.
- The specific importance of the food for the claimed effect. For functions affected by a large number of dietary factors whether a reference to a single food is scientifically pertinent.

In addition, EFSA should consider the claimed effect on the function, and provide advice on the extent to which:

- the claimed effect of the food in the identified function is beneficial.
- a cause and effect relationship has been established between consumption of the food and the claimed effect in humans and whether the magnitude of the effect is related to the quantity consumed.
- where appropriate, the effect on the function is significant in relation to the quantity of the food proposed to be consumed and if this quantity could reasonably be consumed as part of a balanced diet.
- the specific study group(s) in which the evidence was obtained is representative of the target population for which the claim is intended.

- the wordings used to express the claimed effect reflect the scientific evidence and complies with the criteria laid down in the Regulation.

When considering these elements EFSA should also provide advice, when appropriate:

- on the appropriate application of Article 10 (2) (c) and (d) in the Regulation, which provides for additional labelling requirements addressed to persons who should avoid using the food; and/or warnings for products that are likely to present a health risk if consumed to excess.

APPENDIX B

EFSA DISCLAIMER

The present opinion does not constitute, and cannot be construed as, an authorisation to the marketing of the food/food constituent, a positive assessment of its safety, nor a decision on whether the food/food constituent is, or is not, classified as foodstuffs. It should be noted that such an assessment is not foreseen in the framework of Regulation (EC) No 1924/2006.

It should also be highlighted that the scope, the proposed wordings of the claims and the conditions of use as proposed in the Consolidated List may be subject to changes, pending the outcome of the authorisation procedure foreseen in Article 13(3) of Regulation (EC) No 1924/2006.

APPENDIX C

Table 1. Main entry health claims related to bonito protein, including conditions of use from similar claims, as proposed in the Consolidated List.

ID	Food or Food constituent	Health Relationship	Proposed wording
1716	Bonito protein peptide	Natural blood pressure support	Natural Blood Pressure Support" "Provides peptides isolated from bonito to support healthy blood pressure levels." "Provides effective levels of well-researched, specific peptides for healthy blood pressure support." "Formulated to promote healthy blood pressure levels already within the normal range" "A natural approach for blood pressure support" "Bioactive peptides isolated from bonito fish support blood pressure health naturally." "Testing for LKPNM assures efficacy to help maintain blood pressure already within the normal range."
	Conditions of use – In papers by Fujia: 3 g/day of regular "Katsuobusi Oligopeptide (KO)" or 1.5 g/day of stronger KO (s-KO).		

GLOSSARY AND ABBREVIATIONS

ACE	Angiotensin-converting enzyme
IC50	Inhibitory Concentration 50
KO	Katsuobusi Oligopeptide
LKPNM	Leucine-Lysine-Proline-Asparagine-Methionine
SHR	Spontaneously hypertensive rats
S-type KO	Stronger KO