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(54) Titre : SUPPRESSEUR DE L'ANEMIE ET COUPE-FAIM
(54) Title: SUPPRESSOR OF ANEMIA AND APPETITE SUPPRESSOR

(57) Abrégé/Abstract:
The present invention provides a method for suppressing hemolytic anemia by selectively ameliorating reticulocyte increase and iron deposition on spleen as the side effects of methionine and particularly, an appetite suppressor with reduced such side effects, where threonine is used as the effective ingredient of the suppressor of hemolytic anemia due to methionine and a combination of methionine and threonine is used as the effective ingredient of the appetite suppressor.

ABSTRACT OF THE DISCLOSURE

The present invention provides a method for suppressing hemolytic anemia by selectively ameliorating reticulocyte increase and iron deposition on spleen as the side effects of methionine and particularly, an appetite suppressor with reduced such side effects, where threonine is used as the effective ingredient of the suppressor of hemolytic anemia due to methionine and a combination of methionine and threonine is used as the effective ingredient of the appetite suppressor.

SUPPRESSOR OF ANEMIA AND APPETITE SUPPRESSOR

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to a novel suppressor of anemia and a novel appetite suppressor. More specifically, the present invention relates to a threonine-containing agent for the prophylaxis (prevention), amelioration (improvement) and/or therapeutic treatment of anemia due to methionine; an appetite suppressor containing methionine with an action to suppress appetite and threonine suppressing the action of methionine to elicit anemia; and a feed, nutritious supplement, food or drink or pharmaceutical agent, where the methionine action to elicit hemolytic anemia is ameliorated (improved).

Description of the Related Art

Methionine belongs to a group of sulfur-containing amino acids and is one of the essential amino acids for humans. Methionine is nutritionally important. It has been known that methionine is contained abundantly in animal-derived proteins but is contained less in plant-derived proteins. Further, methionine is medicinally used in amino acid infusion and general amino acid formulations in blend with other essential amino acids, therapeutic agents of liver diseases such as chronic or acute hepatitis and liver cirrhosis and detoxication

agents of chemicals, owing to the anti-fatty liver action or detoxication action (see "General Review of Amino Acid Industry (Aminosan Kogyo no Zenyou)", CMC, pp. 29 - 39, 1988).

It has been known that a large dose of methionine has an action to suppress body weight increase or to decrease body weight owing to appetite loss (appetite lowering), as well as influences (actions) of the occurrence of hemolytic anemia with main symptoms such as reticulocyte increase and iron deposition on spleen, and the like. Such actions are problematic for use in the pharmaceutical field and the field of food products.

So as to overcome the problems (for the attenuation of these methionine actions), Muramatsu (see "Journal of Japanese Society of Nutrition and Food Science (Nihon Eiyo Shokuryo Gakkai Shi)", 37, 399-418, 1984) and Sugiyama et al. (see "J. Nutr. Sci. Vitaminol.", 33, 195-205, 1987) have proposed methods for ameliorating symptoms involved in hemolytic anemia and appetite loss (appetite lowering), using serine and glycine in combination with methionine. However, no report has been issued yet about any method for selectively attenuating such actions. Therefore, a development thereof is requested.

SUMMARY OF THE INVENTION

Purpose of the Invention

It is a purpose of the present invention to provide a pharmaceutical agent capable of preventing, ameliorating

and/or therapeutically treating anemia due to methionine, namely a suppressor of anemia. It is the other purpose of the present invention to provide a pharmaceutical agent with an action to suppress appetite, while selectively suppressing an action eliciting anemia.

Means to achieve the Purposes

The present inventors have made investigations so as to solve the problems and to achieve the above purposes. Then, the inventors have found that although the two methionine actions eliciting hemolytic anemia and appetite loss have been considered to work in combination, the methionine action causing anemia, particularly hemolytic anemia with symptoms including reticulocyte increase and iron deposition on spleen can be suppressed selectively and effectively by appropriate intake or dosing of threonine. Further, the inventors have found that concerning the hemolytic anemia and appetite loss induced by the intake or dosing of methionine, the intake or dosing of threonine in combination with methionine can selectively suppress the methionine action to the above anemia without any influence on the appetite-suppressing action of methionine and that the L-form of threonine has a great safety profile on use and can be used in foods and drinks. Based on these various findings, the present invention has been achieved.

More specifically, a first aspect of the present invention relates to a suppressor of anemia due to methionine, where the suppressor characteristically contains (comprises) threonine, which may or may not be in a salt form. From the standpoint of optical isomer, threonine of L-form metabolizable in biological organisms may be used.

Further, the suppressor of anemia in accordance with the present invention can prevent, ameliorate and/or therapeutically treat anemia due to methionine, specifically hemolytic anemia, particularly reticulocyte increase and iron deposition on spleen.

Thus, the suppressor of anemia in accordance with the present invention may satisfactorily contain a substance with an action inducing hemolytic anemia, for example methionine in the pharmaceutical dosage form thereof. Otherwise, the suppressor of anemia may satisfactorily be in a dosage form of threonine alone. In this case, methionine may or may not be in a salt form. From the standpoint of optical isomer, additionally, naturally occurring L-form is preferable but other isomers such as D-form may also be used. Alternatively, other amino acids may be used in combination. Meanwhile, the suppressor of anemia may contain serine and glycine with an action to suppress anemia, but these are generated via the metabolism of threonine in biological organisms. Hence, these are almost unnecessary.

In accordance with one aspect of the present invention there is provided a composition for suppressing anemia due to methionine, comprising: threonine, or pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier, wherein serine and glycine are absent from the composition.

In accordance with another aspect of the present invention there is provided an appetite suppressor comprising methionine and threonine, or pharmaceutically acceptable salts thereof, and a pharmaceutically acceptable carrier.

In accordance with yet another aspect of the present invention there is provided a feed, nutritious supplement, food or drink, or pharmaceutical agent containing methionine, and further comprising threonine to ameliorate hemolytic anemia due to methionine.

In accordance with still another aspect of the present invention there is provided a use of threonine or pharmaceutically acceptable salt thereof, in the manufacture of a medicament for suppressing anemia due to methionine in a living subject, wherein serine and glycine are absent from the medicament.

In accordance with a further aspect of the present invention there is provided a use of threonine and methionine or pharmaceutically acceptable salts thereof in the manufacture of a medicament for suppressing appetite in a living subject.

In accordance with yet a further aspect of the present invention there is provided a use of threonine or pharmaceutically acceptable salt thereof as a methionine induced anemia suppressing agent.

In accordance with still a further aspect of the present invention there is provided a use of methionine and threonine or pharmaceutically acceptable salts thereof as an appetite suppressing agent.

For the intake or dosing of the suppressor of anemia in accordance with the present invention, the suppressor of anemia can be incorporated or dosed in any form with no specific limitation. The suppressor of anemia is orally given or dosed in a simple manner.

The subject to be given or dosed is an animal utilizing methionine in particular, including for example humans. The subject preferably includes humans. The intake or dose of methionine in this case is preferably about (approximately) 25 to 100 mg/kg/day, more preferably about 25 to 85 mg/kg/day and further more preferably about 30 to 50 mg/kg/day on the basis of the free form thereof. The intake or dose of threonine is preferably about (approximately) 20 to 300 mg/kg/day, more preferably about 20 to 255 mg/kg/day and further more preferably about 30 to 80 mg/kg/day on the basis of the free form thereof.

Alternatively, the content of threonine to methionine is preferably at about (approximately) 80 to 300 % (by weight) and more preferably about 100 to 160 % (by weight) on the basis of the free form thereof.

The form of the suppressor of anemia is not specifically limited to any form. Preferably, the suppressor of anemia is in the forms of feeds, nutritious supplements, drinks and foods (the generic name of drink products and food products) or pharmaceutical agents.

Further, the other aspect of the present invention relates to an appetite suppressor characteristically containing (comprising) methionine and threonine. That is, because threonine never exerts any effect on appetite loss caused by one of the methionine actions, an appetite suppressor containing methionine as the effective ingredient and threonine blended therein can be produced. In this case, methionine may or may not be in a salt form. Similarly, threonine may or may not be in a salt form. Concerning their optical isomers, further, the same as in the suppressor of anemia is true with the appetite suppressor.

Methionine incorporated or dosed at 25 mg/kg/day or more may possibly induce an action to suppress appetite. If at least one of serine and glycine is contained therein, the action to suppress appetite is attenuated (improved) or treated. Hence, these two types of amino acids should never be contained therein.

When the appetite suppressor of the present invention is to be incorporated (given) or dosed, the form of the appetite suppressor is not specifically limited. However, the appetite suppressor is orally incorporated (given) or dosed in a simple manner.

The subject to be given or dosed preferably includes humans or pet animals. In this case, the intake or dose of methionine is preferably about (approximately) 25 to 100

mg/kg/day, more preferably about 25 to 85 mg/kg/day and further more preferably about 30 to 50 mg/kg/day on the basis of the free form thereof. The intake or dose of threonine is preferably about (approximately) 20 to 300 mg/kg/day, more preferably about 20 to 255 mg/kg/day and further more preferably about 30 to 80 mg/kg/day on the basis of the free form thereof.

Alternatively, the content of threonine to methionine is preferably at about (approximately) 80 to 300 % (by weight) and more preferably about 100 to 160 % (by weight) on the basis of the free form thereof.

The form of the appetite suppressor is not specifically limited to any form. Preferably, the appetite suppressor is in the forms of feeds, nutritious supplements, drinks and foods or pharmaceutical agents. Additionally, the appetite suppressor may be in the forms of diet foods or specific health foods.

Another embodiment of the present invention is a methionine-containing feed, nutrient supplement, food or drink or pharmaceutical agent, which characteristically contains (comprises) threonine to ameliorate (improve) hemolytic anemia due to methionine.

In accordance with the present invention, threonine is used in combination, so as to suppress the above described action of methionine having been used conventionally. As to

the method of the use and the like, the descriptions of the above described two embodiments of the present invention can be referenced.

In addition, the following embodiments are also contained in the present invention, as other aspects thereof.

A method for suppressing anemia due to methionine, which comprises administrating threonine, which may or may not be in a salt form, to a living subject.

A method for suppressing appetite, which comprises administrating methionine and threonine to a living subject, where methionine may or may not be in a salt form and threonine also may or may not be in a salt form.

Use of threonine, which may or may not be in a salt form, in the preparation of a suppressor of anemia due to methionine.

Use of methionine and threonine in the preparation of an appetite suppressor, where methionine may or may not be in a salt form and threonine also may or may not be in a salt form.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 comparatively shows reticulocyte counts in rat blood among the individual experimental diet groups in Example 1 (mean $\pm$ SD; n = 6).

Fig.2 comparatively shows heme iron contents in rat spleen among the individual experimental diet groups in Example 2 (mean $\pm$ SD; n = 6).

Regarding the symbols: a, b and C in the above figures, it shows that among or between groups with different symbols of "a", "b" and "c", there is a statistical significant difference (level of significance: 5%).

DETAILED DESCRIPTION OF THE INVENTION

Embodiments of the present invention are now described in more detail hereinbelow.

The present invention encompasses a number of embodiments, namely a suppressor of anemia due to methionine, an appetite suppressor, and a feed, a nutritious supplement, a food or drink, or a pharmaceutical agent, where hemolytic anemia due to methionine is ameliorated, and also some other embodiments thereof described above.

(Suppressor of anemia in accordance with the Present Invention)

The suppressor of anemia in accordance with the present invention is a pharmaceutical agent for suppressing anemia due to methionine, particularly hemolytic anemia, namely hemolytic anemia with symptoms such as reticulocyte increase and iron deposition on spleen.

Reticulocyte means juvenile erythrocyte. The increase of reticulocyte indicates the presence of a phenomenon of vigorous erythrocyte regeneration, namely the presence of anemia or anemia tendency. Further, the activation of

abnormal erythrocyte damage in spleen following the increase of abnormal erythrocyte, which is the etiology of erythrocyte decrease, may sometimes deposit iron derived from abnormal erythrocyte on spleen.

Methionine has traditionally been used in pharmaceutical uses, for example an ingredient of amino acid infusions and general amino acid dosage forms. Methionine is also used for dosage forms to maintain or control liver functions, via the anti-fatty liver action and is additionally used for the detoxication of poisoning chemicals, via the detoxication action. Among these uses, methionine when dosed singly without any combination with other amino acid components (amino acid(s)) with effects on the reduction of the side effects may potentially cause the occurrence of actions (side effects) such as reticulocyte increase and iron deposition on spleen. So as to suppress such side effects in accordance with the present invention, threonine preliminarily blended homogenously with methionine in a methionine dosage form (preparation) can selectively and effectively prevent these side effects. Various foods and drinks prepared by active use of the methionine ingredient (methionine or the like) have been known. The combined use of threonine as described above can bring about the same effect.

Alternatively, threonine has an action to suppress anemia due to methionine, with no or almost no emergence of

any new side effects. Thus, threonine can suppress the side effect described above selectively and effectively. Hence, threonine is promising for use as a suppressor of anemia due to methionine in the field of feeds, nutritious supplements, foods and drinks and pharmaceutical products, and particularly where methionine is used.

The suppressor of anemia due to methionine in accordance with the present invention is not specifically limited to any form for human intake or human dosing. Accordingly, various dosage forms such as oral intake, oral dosing or parenteral dosing (parenteral administration) (intravenous dosing and the like) are possibly employed. From the respect of simplicity, oral intake or oral dosing is preferable. Because the suppressor of anemia can be used in mixture in a product using methionine in a simple manner, the product using methionine can be referenced.

The suppressor of anemia in accordance with the present invention can be used in a dosage form containing methionine and threonine and may satisfactorily contain various formulation substances pharmacologically acceptable (as auxiliary substances) (which may be referred to as "pharmaceutically acceptable carriers" hereinbelow). The formulation (preparation) substances can be selected appropriately, depending on the dosage form of the suppressor of anemia. The formulation substances may be for example

excipients, diluents, additives, disintegrators (disintegrants), binders, coating agents, lubricants, smoothing agents, lubricant pharmaceuticals (lubricants), flavoring agents, sweeteners, solubilization agents and the like. Further, the formulation substances specifically include for example magnesium carbonate, titanium dioxide, lactose, mannitol and other sugars, talc, milk protein, gelatin, starch, cellulose and derivatives thereof, animal and vegetable oils, polyethylene glycol, and solvents, for example sterile water and monovalent or polyvalent alcohols, for example glycerol.

The suppressor of anemia in accordance with the present invention can be prepared in various pharmaceutical dosage (preparation) forms known as described above or to be possibly developed in future, for example various dosage forms for oral dosing, intraperitoneal dosing, transdermal dosing, and inhalation dosing. So as to prepare the pharmaceutical agent of the present invention into these various pharmaceutical dosage forms, methods having been known or to be possibly developed in future can appropriately be employed.

These various pharmaceutical dosage forms (including nutritious supplements) include for example dosage forms in appropriate solids or liquids, which are for example granules, powders, coated tablets, tablets, (micro)capsules, suppositories, syrups, juices, suspensions, emulsions,

infusions (dropping agents), solutions for injections, and dosage forms of sustained release types of active substance.

As for quantities of the methionine and threonine used, it is needless to say that the suppressor of anemia in one of the dosage forms described above in accordance with the present invention should contain the above described ingredients (methionine and threonine) at quantities effective for the exertion of their pharmaceutical efficacies.

The subject to be given or dosed includes animals utilizing methionine in particular, for example humans. The subject preferably includes humans. The intake or administration dose of methionine in this case is preferably about (approximately) 25 to 100 mg/kg/day, more preferably about 25 to 85 mg/kg/day and further more preferably about 30 to 50 mg/kg/day on the basis of the free form thereof. The intake or administration dose of threonine is preferably about (approximately) 20 to 300 mg/kg/day, more preferably about 20 to 255 mg/kg/day and further more preferably about 30 to 80 mg/kg/day on the basis of the free form thereof.

Alternatively, the content of threonine to methionine is preferably at about (approximately) 80 to 300 % (by weight) and more preferably about 100 to 160 % (by weight) on the basis of the free form thereof.

Embodiments of its application to pharmaceutical products have been described above. Based on the embodiments,

the inventive suppressor is easily applicable to feeds, nutritious supplements, and foods and drinks. Generally, threonine is added for the purpose of suppressing the above described side effects in methionine products. The object product can be obtained or recovered at a state such that a given quantity of threonine is homogenously mixed with methionine.

(Appetite suppressor of the Present Invention)

An appetite suppressor is now described as a second embodiment of the present invention.

In accordance with the present invention, methionine-induced hemolytic anemia involving symptoms such as reticulocyte increase and iron deposition on spleen as described above can be suppressed by using methionine and threonine in combination, without any influence on the methionine action to suppress appetite. The action is effectively utilized in accordance with the present invention.

The appetite suppressor of the present invention will now be described in more detail hereinbelow.

As described above, methionine when used singly in various products exerts its actions such as reticulocyte increase and iron deposition on spleen, as well as an action to suppress appetite. Threonine preliminarily contained together with methionine in such product can suppress

selectively and effectively the action causing anemia. In other words, the threonine action to suppress anemia together with the methionine action to suppress appetite can singly be utilized in an effective manner.

Alternatively, threonine exerts its action to suppress anemia selectively and effectively when threonine is contained together with methionine as described above. Additionally, the resulting formulation can also suppress appetite with no or almost no emergence of other side effects. Thus, the combined use of methionine and threonine is quite promising for an appetite suppressor.

Like the suppressor of anemia, the appetite suppressor of the present invention may be used in the form of a pharmaceutical product (including nutritious supplements) and may contain various formulation substances pharmacologically acceptable (as auxiliary substances) (which may be also referred to as "pharmaceutically acceptable carriers"). The formulation substances may be appropriately selected, depending on the dosage form of the formulation (preparation) thereof. The formulation substances may be for example excipients, diluents, additives, disintegrators, binders, coating agents, lubricants, smoothing agents, lubricant pharmaceuticals (lubricants), flavoring agents, sweeteners, and solubilization agents. Further, the formulation substances are specifically for example magnesium carbonate,

titanium dioxide, lactose, mannitol and other sugars, talc, milk protein, gelatin, starch, cellulose and derivatives thereof, animal and vegetable oils, polyethylene glycol, and solvents, for example sterile water and monovalent or polyvalent alcohols, for example glycerol.

These various pharmaceutical dosage (preparation) forms include for example dosage forms in appropriate solids or liquids, which are for example granules, powders, coated tablets, tablets, (micro)capsules, suppositories, syrups, juices, suspensions, emulsions, infusions (dropping agents), solutions for injections, and dosage forms sustaining the release of an active substance.

As for quantities of methionine and threonine, it is needless to say that the appetite suppressor in one of the dosage forms described above in accordance with the present invention should contain the ingredients (methionine and threonine) at quantities effective for the exertion of their pharmaceutical efficacies.

The application of the present invention to pharmaceutical products has been described above. Methionine combined with threonine when used in foods and drinks can exert the effects described above. Accordingly, the present invention is expected to be applicable to these foods and drinks, particularly those using methionine.

For the appetite suppressor of the present invention,

embodiments of various nutritious supplements, foods and drinks or pharmaceutical products having been known or being possibly developed in future can be selected appropriately. Embodiments of diet foods or specific health food products can be also selected.

The embodiments for various foods and drinks and the like as the appetite suppressors in various forms (products) include for example sweets (cool sherbet, jelly, cake, candy), bread, chewing gum and products for animals (pet) except for humans. In such manner, embodiments of products with the additional appetite-suppressing action are also encompassed within the scope of the present invention.

The subject to which the appetite suppressor of the present invention is given or dosed is not specifically limited but preferably includes humans and pet animals. The form of the intake or dosing in this case is not specifically limited. Thus, various dosing forms may be selected, including for example oral intake, oral dosing, and parenteral dosing (intravenous dosing and the like). From the standpoint of simplicity, oral intake or oral dosing is preferable. It has been known that methionine has an action to suppress appetite and the use of methionine for the purpose has also been known. For the known use of methionine, threonine is homogenously mixed with methionine for use in combination.

The intake or dose of methionine in this case is

preferably about (approximately) 25 to 100 mg/kg/day, more preferably about 25 to 85 mg/kg/day and further more preferably about 30 to 50 mg/kg/day on the basis of the free form thereof. The intake or dose of threonine is preferably about (approximately) 20 to 300 mg/kg/day, more preferably about 20 to 255 mg/kg/day and further more preferably about 30 to 80 mg/kg/day on the basis of the free form thereof.

Alternatively, the content of threonine to methionine is preferably at about (approximately) 80 to 300 % (by weight) and more preferably about 100 to 160 % (by weight) on the basis of the free form thereof.

Based on the descriptions above, apparently, the inventive appetite suppressor containing a combination of threonine and methionine can selectively and effectively suppress the action of methionine causing anemia, so that only the appetite-suppressing action can be effectively utilized. Hence, the pharmaceutical agent can readily be used practically.

(Nutritious supplements, foods and drinks or pharmaceutical products of the present invention)

An additional embodiment of the present invention is a methionine-containing feed, nutritious supplement, food or drink or pharmaceutical product, which characteristically contains (comprises) threonine and can ameliorate (improve)

hemolytic anemia due to methionine.

The methods for using methionine and threonine are as described above in the two embodiments. Therefore, it is not at all difficult to practice the present invention.

For example, threonine (at a given amount) can be homogeneously mixed with methionine for use in methionine-containing known feeds, nutritious supplements, foods and drinks or pharmaceutical products, particularly in which it is intended to ameliorate the side effects of methionine.

As described above, the inventive products of feeds, nutritious supplements, foods and drinks or pharmaceutical products in which hemolytic anemia due to methionine is ameliorated can readily be produced or obtained, utilizing the descriptions in the two embodiments of the present invention and known techniques.

The appetite loss or the suppression of body weight increase owing to a large dose of methionine has been known but the expression mechanism therefor has scarcely been known yet. A possibility cannot yet totally be eliminated that the rejection of the intake of methionine diet per se may directly lead to the emergence of these phenomena. The reason is that experimental animals at almost all the research tests were fed with experimental feeds in blend with methionine so the animals could not select experimental diets except for the methionine feeds. When animals happen to reject intake of such methionine

diets, the animals may possibly reject the odor or taste of methionine per se or the odor or taste thereof occurring synergistically with other ingredients in the feeds. Further, it has been suggested that threonine does not have a suppressive action of appetite or body weight increase so strong as that of methionine (see Muramatsu, Journal of Japanese Society of Nutrition and Food Science (Nihon Eiyo Shokuryo Gakkai Shi), 37, 399-418, 1984).

So as to verify the findings so far, therefore, simultaneous feeding experiments with two types of methionine diet and threonine diet were done as described below (see Example 5). The effect of methionine intake was verified.

The Japanese Patent Application No. 2001-165242, filed on May 31, 2001, based on which the priority is claimed for this application, is incorporated therein by reference in its entirety.

DESCRIPTION OF PREFERRED EMBODIMENTS

The present invention will be described in more detail in the following examples. However, the present invention is not limited to these Examples.

(Example 1)

Suppressive effect on reticulocyte increase

(Experimental Animals)

Male F344/DuCrj rats of age 4 weeks were purchased from

Charles River Japan, Inc. The rats were placed and fed in bracket cages in environment at room temperature of about 25 °C and a relative humidity of about 60 % in a bright/dark cycle where the bright stage was set from 7:00 to 19:00. After the animals were preliminarily fed with a commercially available powder feed CRF-1 (manufactured by Oriental Yeast Co., Ltd.) for one week and with the following no-addition diet for one week, 6 rats were randomly assigned per group. Then, the following experimental diets were fed ad libitum for 2 weeks. As drinking water, tap water was fed ad libitum.

(Experimental Feeds)

No-addition diet was composed of β-cornstarch at 62.9 %, casein at 20.0 %, soybean oil at 7.0 %, cellulose at 5.0 %, AIN-93G mineral mix (Oriental Yeast Co., Ltd.) at 3.5 %, AIN-93 vitamin mix (Oriental Yeast Co., Ltd.) at 1.0 %, L-cystine at 0.3 %, choline tartrate at 0.25 % and t-butylhydroquinone at 0.0014 %. The diet was essentially based on the US NRC AIN-93G purified feed composition (1995), but the α-cornstarch and sucrose in the original composition were totally substituted with (replaced by) β-cornstarch. When amino acid was to be contained in an experimental diet, the amino acid was substituted for a part of the β-cornstarch. When methionine or threonine was to be contained in an experimental diet, methionine and threonine were at 2.4 % and 3.8 %, respectively.

When methionine and threonine were to be simultaneously contained in an experimental diet, methionine and threonine were at the same ratios as described above.

(Amino Acids)

All amino acids used were amino acids (L-forms; free forms) manufactured by Ajinomoto Co., Inc. The same is true with the following Examples.

(Various Measurements)

On day 14 after feeding with the individual experimental diets, the animals were anesthetized with ether, after laparotomy, blood was taken from abdominal inferior vena cava. Using blood treated with EDTA-2K for anti-coagulation, blood smear samples were prepared by the Brecher's method (see "Handbook of All Laboratory Test Techniques (Rinshou Kensa Gijyutsu Zensho)", Vol. 3, Laboratory Blood Test, 1972). Reticulocyte was counted with an automatic blood image typing apparatus (Hitachi Type 8200).

Groups with the individual experimental diets were compared together by the Tukey's multiple comparison test. At the level of significance $P < 0.05$, statistical significance was determined.

(Results)

The no-addition diet and the threonine diet were fed to the rats. No increase of reticulocyte was observed. As described in the findings of the related art, the reticulocyte count was distinctly increased in the rats fed with the methionine diet. However, in a group simultaneously dosed with methionine and threonine, the increase of reticulocyte count as one of the indicators of hemolytic anemia was suppressed at almost the same level as in the no-addition group, which was a statistically significant effect (see Fig. 1). This indicates that simultaneous addition of threonine selectively suppresses the side effect of methionine.

(Example 2)

Suppressive effect on iron deposition on spleen

The same experimental animals and experimental diets (feeds) as in Example 1 were used.

On day 14 after the feeding with the individual experimental diets, the animals under anesthesia with ether were exposed to exsanguination from abdominal aorta and led to death, to resect spleen and weigh the weight. Immediately thereafter, the spleen was frozen in liquid nitrogen and stored in an ultra-low freezer.

After pulverizing the resected spleen under cooling in ice with Polytron, the non-heme iron content in the spleen was determined by absorptiometry with o-phenanthroline according

to the Kaldor's method (see Austral. J. Exp. Biol., 32, 795-800, 1954).

The items assayed for the individual groups fed with these experimental diets were compared together by the Tukey's multiple comparison test. At the level of significance $P < 0.05$, statistical significance was determined.

(Results)

Iron deposition on spleen as another indicator of hemolytic anemia was examined. Compared with the group fed with the no-addition diet, the accumulated iron on spleen was distinctly increased in the rats fed with the methionine diet. However, the accumulated iron in the rats fed with the (methionine + threonine) diet was at a higher level than the normal level in the rats fed with the no-addition diet. Compared with the rats fed with the methionine diet, the iron accumulation in spleen in the rats fed with the (methionine + threonine) diet was significantly suppressed (see Fig. 2). This indicates that threonine added to the methionine diet possibly suppressed the onset of hemolytic anemia and consequently suppressed iron deposition on spleen.

(Example 3)

Effect on appetite loss

The same experimental animals were used as in Example

1. The same compositions of the experimental diets as in Example 1 were used. As amino acids to be contained, however, methionine, glycine and threonine were blended singly or in combination with methionine to 2.4 %, 2.4 % and 3.8 %, respectively in experimental diets, by substituting the amino acids for a part of β-cornstarch.

(Various Measurements)

Body weight and feed intake were measured daily. The total body weight increment and total intake over the 2 weeks during feeding with such experimental diets are shown below in the following Table 1.

Table 1

Experimental diet fed	Body weight increment [1, 2] (g/2 weeks)	Experimental diet intake [1, 2] (g/ 2 weeks)
No-addition diet	60.0 ± 5.2 ab	175.9 ± 8.4 a
2.4 % methionine diet	0.2 ± 2.3 e	94.2 ± 4.5 b
2.4 % glycine diet	66.2 ± 8.4 a	184.0 ± 7.9 a
3.8 % threonine diet	56.2 ± 6.3 bc	173.5 ± 5.8 ac
2.4 % methionine + 2.4 % glycine diet	48.4 ± 6.0 c	159.2 ± 10.0 c
2.4 % methionine + 3.8 % threonine diet	12.6 ± 3.9 d	108.5 ± 4.7 b

1.: mean ± standard deviation (SD); n = 6

2.: statistic significance among or between groups with different symbols "a" to "e" (at a 5% level of significance); no statistic significance among groups with identical symbols and with symbols including identical symbols.

(Results)

Suppression of body weight and intake was distinctly demonstrated in the rats fed with the methionine diet. Based on the similar changes of the suppression of body weight increase and the suppression of intake among the experimental diets groups, it is indicated that the suppression of body weight increase may be caused by the suppression of intake and that the suppression of intake may potentially be caused by appetite loss. Alternatively, such influences were never observed in the groups fed with the experimental diets singly blended with glycine or threonine. Further, the (methionine + glycine) diet was fed to the rats. Then, the influence observed in the rats fed with the methionine diet was greatly ameliorated (improved) in the groups fed with the (methionine + glycine) diet. In the (methionine + threonine) diet-fed group [(methionine + threonine) group], the body weight increase was improved at some extent, compared with the methionine diet-fed group. However, almost no change was observed in the intake between the (methionine + threonine) group and the methionine diet-fed group (methionine group). It is thought that based on these results, the effect of simultaneously ingested threonine on the suppression of appetite loss occurring due to a large excess intake of methionine is weaker than that of glycine or serine.

As described above, apparently, simultaneous intake of threonine with methionine is effective for selectively

reducing the onset of the side effect induced by methionine intake, namely hemolytic anemia. Accordingly, such simultaneous threonine intake therewith allows the selective application of the methionine function, namely the action to decrease appetite.

(Example 4)

Effect on appetite loss in different animal species and at senile stage

(Experimental Animals)

Male Crj:CD(ICR) mice of age 8 weeks were purchased from Charles River Japan, Inc.. The mice were placed and fed in polycages in environment at room temperature of about 25 °C and a relative humidity of about 60 % in a bright/dark cycle where the bright stage was set from 7:00 to 19:00. Up to age 87 weeks, the mice were fed with a commercially available solid feed CRF-1 (manufactured by Oriental Yeast Co., Ltd.), to promote the senility of the mice. Immediately before the age 88 weeks, the animals (mice) were assigned to two groups, namely a group with no-addition diet (n = 25) and a group fed with an experimental diet [(methionine + threonine) diet] (n = 27). The experimental diet was as follows. The experimental diet was fed for 2 weeks. During the period, 4 animals of the no-addition diet group died, while 3 animals of the (methionine + threonine) diet group died. Autopsy findings suggested that

all the animals possibly ended in natural death. Finally, n = 21 in the no-addition diet group and n = 24 in the (methionine + threonine) diet group were made. As drinking water, tap water was fed ad libitum.

(Experimental Feeds)

The no-addition diet and the experimental diet [(methionine + threonine) diet] were prepared in the same manner as in Example 1. Because the weight of mouse is considerably less than the weight of rat, smaller 2-% quantities of both methionine and threonine were blended, because of the difference in body weight.

(Various Measurements)

Body weight and feed intake were measured. Table 2 shows body weight, total body weight increment, and total intake on week 2 from the start of the feeding of the experimental diet. Herein, the groups were compared together by the Student's t test in terms of individual measured items. At the level of significance, $P < 0.05$, statistic significance was determined.

Table 2

Experimental diet fed	Body weight at start of feeding (1,2) (g)	Body weight on week 2 from start of feeding (1,2) (g)	Body weight increment (1, 2) (g/2 weeks)	Intake of experimental diet (1,2) (g/2 weeks)
No-addition diet	49.8 ± 5.6 a	49.2 ± 7.2 a	-0.6 ± 2.9 a	65.6 ± 8.3 a
2 % methionine + 2 % threonine diet	49.4 ± 4.2 a	43.7 ± 5.3 b	-5.7 ± 3.9 b	48.5 ± 9.6 b

1.: Numerical figure shows mean ± SD in the table.

2.: There is a statistic significance between different symbols "a" and "b" (at a 5% level of significance); and no significant difference between identical symbols.

(Results)

2 weeks later after the start of feeding with the experimental diet, all of the body weight, body weight increment and experimental diet intake were significantly reduced in the (methionine + threonine) group. This indicates that the effect of the (methionine + threonine) blend diet on appetite loss as certified at the experiment about rats of aged 6 to 8 weeks in Example 1 is apparently exerted effectively in the different animal species (mice) and at senile stage (88 to 90 weeks old).

(Example 5)

Verification of effect in methionine intake

(Experimental Animals)

Male F344/DuCrj rats of age 6 weeks (5 animals; Charles River Japan, Inc.) preliminarily fed with a commercially available powder feed CRF-1 (Oriental Yeast Co., Ltd.) for one week were used. The same environmental conditions as described in Example 1 were used for the room temperature, humidity and bright and dark cycle of the feeding environment.

(Experimental Feeds)

As described above in Example 1, a part of β-cornstarch in the experimental diet based on the AIN-93G composition (US NRC, 1995) was replaced with (by) methionine or threonine, so as to blend each of the amino acids in the experimental diet. The blend ratios of methionine and threonine in the resulting experimental diet were at 2.4 % and 3.8 %, respectively.

(Experimental Method)

Two feeding boxes were arranged per one rat. One of the boxes contained the methionine diet, while the other contained the threonine diet. These two feeding boxes were simultaneously given to the individual rats, to measure body weight and feed intake daily. The measurement was continued for 2 weeks.

(Results)

Table 3 below shows total body weight increment, total methionine diet intake, total threonine diet intake and total diets intake per individual (animal) over 2 weeks of feeding with the individual experimental diets.

Table 3

Animal No.	Experimental diet intake (g/ 2 weeks)			Body weight increment (g/ 2 weeks)
	2.4 % methionine diet	3.8 % threonine diet	total	
1	11.2	80.9	92.1	28.6
2	47.7	1.7	49.4	-5.9
3	43.4	1.3	44.7	-6.5
4	7.1	83.2	90.3	29.3
5	44.5	1.2	45.7	-8.4

After the 2-week feeding with the individual experimental diets, 3 of the 5 animals selectively ingested the methionine diet, while the remaining 2 animals favored the threonine diet. Compared with the 2 animals favoring the threonine diet, additionally, the body weight increase of the 3 animals having selectively ingested the methionine diet had been suppressed apparently. This possibly indicates that the appetite loss or the suppression of body weight increase having been known as an action of a large dose of methionine is not an effect directly ascribed to the rejection of the intake of the methionine diet but a secondary action occurring in the biological organisms after methionine intake. Hence, the development of an appetite suppressor using methionine is highly possible in practical sense.

(Example 6)

Production of methionine dosage form

For the production and dosing (the administration) of

a methionine dosage form, it is the simplest way to mix together DL-methionine (1.0 g) and L-threonine (1.3 g) and seal the resulting mixture in a capsule. Besides, a method for producing a methionine tablet is suggested, including a step for adding powders or excipients to the resulting mixture. So as to obtain or recover the effect of appetite loss from these dosage forms (preparations), these dosage forms may be given, for example three times daily, 30 to 60 minutes before meals.

(Example 7)

Production of diet food

Table 4 below shows a composition as one example of a dietary food (diet food) complying with the Recommended Diet Allowances Standard in the dosage three times daily and having a possible effect on appetite loss (appetite lowering) toward foods other than the dietary food.

Table 4

Composition for use in dietary food (energy: 800 kcal per one meal)

DL-Methionine	1.0 g
L-Threonine	1.3 g
Protein	23.6 g
Lipid	17.8 g
Carbohydrate	134.1 g
Sodium	867 mg
Potassium	667 mg
Calcium	200 mg
Iron	3.3 mg
Magnesium	83 mg
Phosphorus	233 mg
Vitamin A	600 IU
Vitamin B1	0.3 mg
Vitamin B2	0.3 mg
Vitamin B6	0.4 mg
Vitamin B12	0.8 μg
Niacin	4.3 mg
Pantothenic acid	1.7 mg
Folic acid	67 μg
Vitamin C	33 mg
Vitamin D	33 mg
Vitamin E	2.7 mg
Dietary fiber	6.7 g

Foods in the forms of drinks and sweets and other forms are exemplified, which can be readily ingested orally and can be packed in portable wrapping materials. Further, the addition of flavoring agents, sweeteners, spices and the like is also exemplified so as to permit easy oral ingestion.

Effect of the Invention

In accordance with the present invention, a suppressor of anemia containing (comprising) threonine as an effective ingredient, preferably a suppressor of hemolytic anemia due

to methionine is provided. In addition, an appetite suppressor containing (comprising) a combination of methionine and threonine as effective ingredients can be provided as well.

Because, in particular the significant side effects of methionine namely reticulocyte increase and iron deposition on spleen can be ameliorated (reduced) selectively, the use of methionine having been restricted in the fields of nutritious supplements, pharmaceutical products and foods and drinks due to the side effects will possibly be enlarged from the respects of volume and applicability with expectation. Particularly, the inventive product is highly promising as a safe appetite suppressor with reduced hemolytic anemia.

CLAIMS:

1. A composition for suppressing anemia due to methionine, comprising:

threonine, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier,

wherein serine and glycine are absent from the composition.

2. The composition according to claim 1, wherein the anemia is hemolytic anemia.

3. The composition according to claim 1 or 2, wherein the composition at least one of prevents and ameliorates the anemia by increasing reticulocytes and iron deposition on the spleen.

4. An appetite suppressor comprising methionine and threonine, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

5. The appetite suppressor according to claim 4, wherein serine and glycine are absent.

6. The appetite suppressor according to claim 4 or 5, wherein the appetite suppressor is for humans and in a methionine dosage form adapted to provide 25 to 100 mg/kg/day on the basis of the free form thereof and in a threonine dosage form adapted to provide 20 to 300 mg/kg/day on the basis of the free form thereof.

7. The appetite suppressor according to any one of claims 4 to 6, comprising a weight percentage of threonine to methionine of 80 to 300% by weight on the basis of the free form thereof.

8. A feed, nutritious supplement, food or drink, or pharmaceutical agent containing methionine, and further comprising threonine to ameliorate hemolytic anemia due to methionine.

9. Use of threonine or pharmaceutically acceptable salt thereof, in the manufacture of a medicament for suppressing anemia due to methionine in a living subject,

wherein serine and glycine are absent from the medicament.

10. Use of threonine and methionine or pharmaceutically acceptable salts thereof in the manufacture of a medicament for suppressing appetite in a living subject.

11. The use according to claim 10, wherein serine and glycine are absent from the medicament.

12. The use according to claim 10 or 11, wherein the medicament is for humans and in a methionine dosage form adapted to provide 25 to 100 mg/kg/day on the basis of the free form thereof and in a threonine dosage form adapted to provide 20 to 300 mg/kg/day on the basis of the free form thereof.

13. The use according to any one of claims 10 to 12, wherein the medicament has a weight percentage of threonine to methionine of 80 to 300 % by weight on the basis of the free form thereof.

14. Use of threonine or pharmaceutically acceptable salt thereof as a methionine induced anemia suppressing agent.

15. Use of methionine and threonine or pharmaceutically acceptable salts thereof as an appetite suppressing agent.

FIG. 1

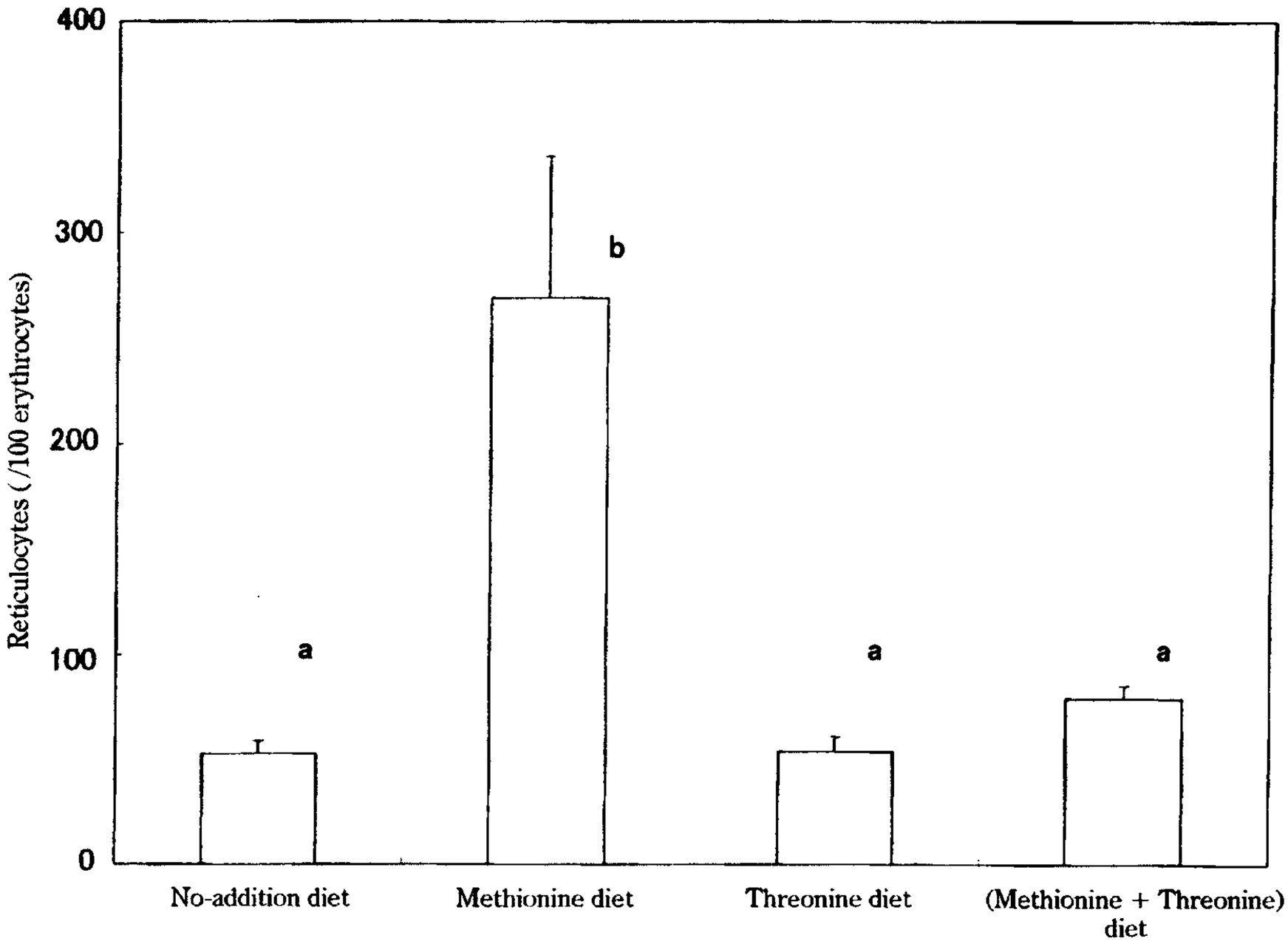
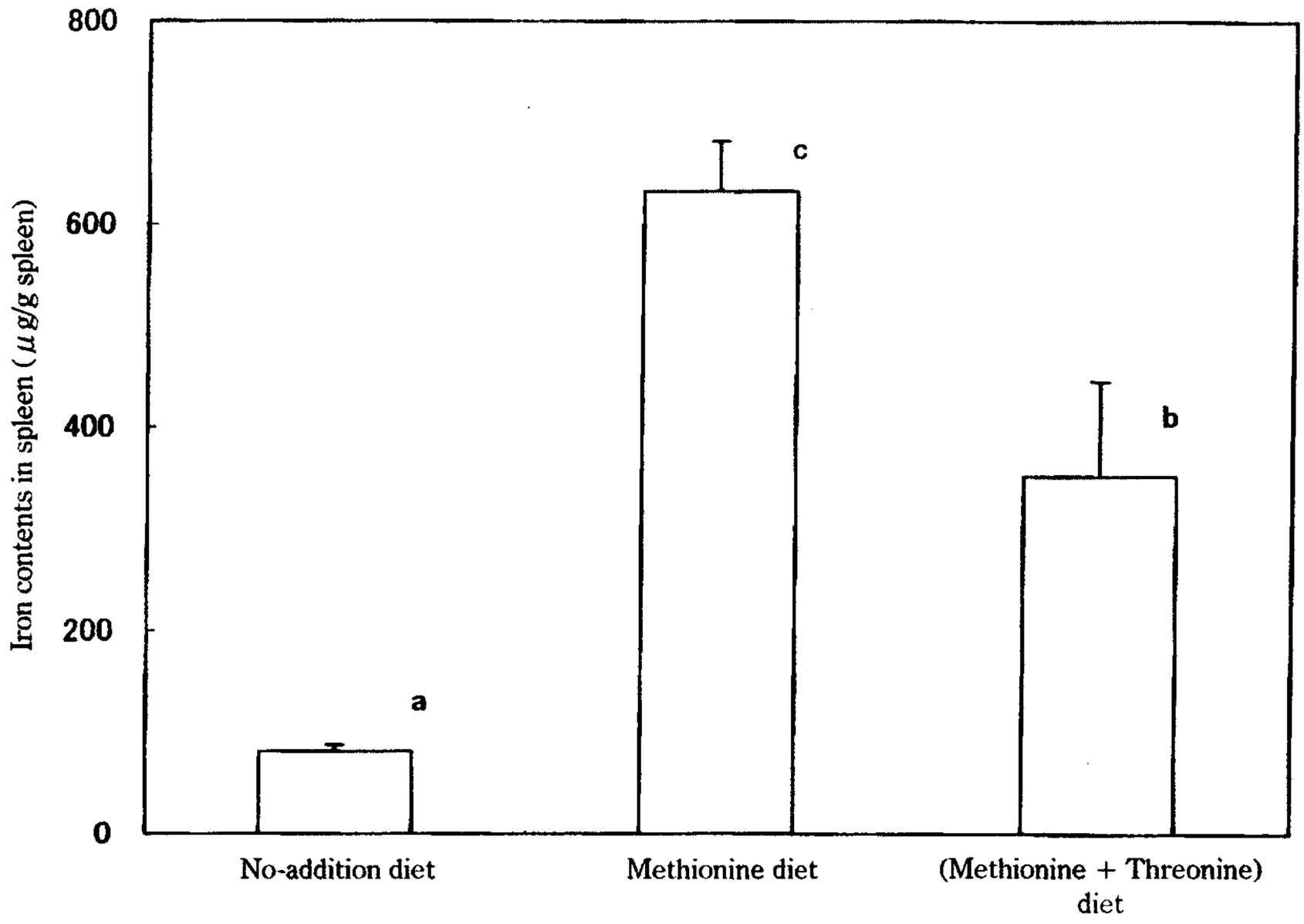

400
300
200
100
0
Reticulocytes (/100 erythrocytes)
a
b
a
a
No-addition diet
Methionine diet
Threonine diet
(Methionine + Threonine) diet

FIG. 2


800
600
400
200
0
Iron contents in spleen (μg/g spleen)
a
c
b
No-addition diet
Methionine diet
(Methionine + Threonine) diet