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**FEED SUPPLEMENT FOR ANIMALS AND  
 PRODUCTION THEREOF**

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**ABSTRACT OF THE DISCLOSURE**

In producing an animal feed supplement wherein a microorganism selected from the group consisting of *Bacillus subtilis* and *Bacillus natto* is cultivated in a culture medium, the improvement of enhancing the growth-promoting activity thereof by stopping the cultivation at a point between the beginning and middle of the logarithmic growth phase of the microorganism, and heating the resulting culture at a pH of 4.0 to 8.0 at a temperature of 50 to 80° C. for 1 to 3 hours. The invention also includes an animal feed supplement produced by the above-described process and an animal feed containing said feed supplement.

This invention relates to a novel feed supplement for animals and also to a process for producing the same. This invention also relates to an animal feed to which such supplement is added.

It has recently come to be conventional to add such nutrients as vitamins, amino acids, antibiotics, enzymes or disease preventives to feeds for animals. In view of the present status of the world-wide development of the livestock raising industry, the development of an effective feed supplement would amount to a significant contribution to the art.

The present invention relates to the preparation of a feed supplement for animals characterized by cultivating a microorganism belonging to *Bacillus subtilis* or *Bacillus natto* preferably by submerged cultivation with vigorous agitation and aeration, stopping the cultivation between the beginning and middle of the logarithmic growth phase of the microorganism and then heat-treating the culture broth under particular conditions so that there may be produced, at a high yield, a highly effective feed supplement which is stable and is useful as an additive for feeds for poultry and animals.

We have found, through extensive research on the effect of fermentation products of microorganisms on animal feeds, that the effect as a feed supplement of the culture product of a microorganism belonging to *Bacillus subtilis* or *Bacillus natto* is not stable and varies depending on the particular method employed for preparing the same. Thus, in some cases, no effect has been observed; in others, a result amounting to the inhibition of the growth of animals has been often observed.

As a result of further investigations and research from various angles in view of these observations, we have discovered that it is important to stop the cultivation at a particular stage as described before and to heat-treat the resulting cultivation product or broth under specific conditions. For example, we have found that, in the case of conventional organisms of *Bacillus subtilis* (known as *Bacillus subtilis* N' strain) widely used in the production of amylase and protease, a substance which will promote or stimulate the growth of animals when administered to them will be produced in the culture solution and cells

within a period far earlier than that period when such enzyme as amylase or protease and antibiotics begin to be accumulated in a large amount. We have further found that, when the cultivation is continued until the growth of the microorganism goes into the latter part of the period of the logarithmic growth phase, the production of the enzyme will still continue but, on the other hand, a factor acting to inhibit the growth of animals will also be produced in the culture solution. That is to say, in the period from the beginning to the middle of the logarithmic growth phase of bacteria, a factor or substance effective for the promotion of the growth of animals will be produced and accumulated both inside and outside the cells (that is, in the culture filtrate); while the production of the factor inhibiting the growth of animals will be negligible.

We have also discovered that, when the culture solution or broth containing the cells obtained in this particular period is heated under selected conditions, the activity of the product to promote the growth of animals will increase.

Microorganisms which are used in carrying out the present invention are those belonging to *Bacillus subtilis* and *Bacillus natto*. As is well known, in the strains belonging to these groups are included such strains producing amylase and protease in a large amount and used industrially as, for example, a *Bacillus subtilis* N strain (Hagihara, 1958), N' strain (Boyer et al., 1960), R strain (Hagihara, 1958), K strain (Oishi et al., 1963), H strain (Nishimura et al., 1959) and *Bacillus natto* Sawamura strain and SN strain. Also included are strains producing the above mentioned enzymes only in slight amounts and used often for genetic research as, for example, Marburg strains No. SB-15 (Nester et al., 1961), No. 160 (Saito et al., 1961), No. 168 (Burkholder et al., 1947), No. 30 (Ephrati-Elizur et al., 1961) and No. W 23 (Thorne, 1962). These strains are well known to those skilled in the art and are easily available from various public culture collections.

According to the present invention, the above described microorganisms are cultivated. Either a solid culture process or submerged culture process with agitation and aeration may be employed. However, the submerged culture process is most preferred due to the fact that various conditions required in carrying out the present invention can be controlled easily and positively.

Any culture medium which is well known in the cultivation of microorganisms of *Bacillus subtilis* and *Bacillus natto* may be employed. For the carbon source can be used starch, corn meal, dextrin, glucose and sucrose. For the nitrogen source can be utilized not only such inorganic nitrogen sources as ammonium chloride NH<sub>4</sub>Cl, ammonium sulfate (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, ammonium phosphate (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub> and ammonium nitrate NH<sub>4</sub>NO<sub>3</sub> but also such beans as soybeans and corn steep liquor, defatted powdered milk, casein and amino acids. Inorganic nutrient sources are also required, for example, K<sub>2</sub>HPO<sub>4</sub> and other auxiliary salts. Industrially preferable is a culture medium consisting of a proper combination of 3 to 20% alkali extract of defatted soybean, 0.5 to 10% corn steep liquor, 1 to 10% starch, 1 to 8% corn meal, 1 to 5% rice bran and 1 to 5% bran with the addition of a small amount of nutrient inorganic salts.

The starting pH of the culture medium is 6.0 to 8.0 or preferably 6.5 to 7.2. The temperature may be 30 to 40° C. or preferably 35 to 38° C.

The culture age is very important to the practice of the invention as mentioned before. Generally the time of the shift from one growth phase to the next one will be remarkably influenced by the particular strain, condition of