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Cordyceps extract as a dietary supplement and uses thereof

Field of the invention

The present invention relates to a dietary supplement comprising extract(s) of *Cordyceps* species. The dietary supplement may be added to feed and/or
5 drinking liquid to improve the health and general well being of animals, particularly for oviparous animals. The invention also relates to the use of the dietary supplement in animal feed and/or drinking liquid to improve the quality of eggs produced.

Background of the invention

10 Animal husbandry is the agricultural practice of breeding and rearing livestock and has a long history. The field of animal husbandry includes the management, handling, nutrition, breeding and welfare of animals. Animals are cultivated for meat and for produce such as wool, milk and eggs. Animals that are commonly reared for these purposes include cattle, goats, sheep, pigs, fowl
15 and poultry.

Research into animal nutrition and animal feed formulation aims to improve the growth, health and wellbeing of animals and the quality of the animal produce. Such animal feed formulations can include complete feeds comprising all the required nutrients, concentrates that provide part of the required nutrients, (such
20 as protein or carbohydrates) or supplements that provide additional micro-nutrients, (for example, vitamins and minerals). The concentrates and supplements can be purchased as separate formulations by the farmer and mixed into animal feed as necessary.

Poultry, in particular fowl, are very important agricultural animals which are reared as food and for their eggs. These most typically are members of the orders Galliformes (such as chickens and turkeys), and Anseriformes (waterfowl such as ducks and geese). Other than rearing poultry for their meat, eggs are an important poultry produce which can be eaten.

Eggs are very nutritious and contain a large quantity of protein and also vitamins and minerals, including vitamin A, riboflavin, folic acid, vitamin B6, vitamin B12, choline, iron, calcium, phosphorus and potassium. However, the egg yolk contains a significant amount of cholesterol, more than two thirds of the recommended daily cholesterol intake. It is the high cholesterol content in eggs and the associated adverse publicity that poses a challenge to egg producers.

Cholesterol is an important precursor in the synthesis of the sex steroid and adrenal hormones and plays a central role in many biochemical processes. The human body is capable of synthesising cholesterol *de novo*, but cholesterol is also consumed in the diet. There is link between high cholesterol levels and cardiovascular diseases, and consumers tend to be concerned about cholesterol in the diet.

It is therefore desirable to have a method of producing eggs with a reduced cholesterol content for the purposes of catering to consumer satisfaction. It would also be beneficial to improve the nutrients of eggs.

Summary of the invention

According to a first aspect, the present invention provides a dietary supplement comprising at least one *Cordyceps* species or at least one extract of at least one *Cordyceps* species. In general, any *Cordyceps* species is contemplated.

The *Cordyceps* species may be selected from the group consisting of *Cordyceps sinensis*, *Cordyceps militaris*, *Cordyceps gracilis*, *Cordyceps ophioglossoides*, *Cordyceps memorabilis*, *Cordyceps sphecocephala*, *Cordyceps hawkesii*, *Cordyceps martialis*, *Cordyceps sobolifera* and *Cordyceps capitata* . The *Cordyceps* extract may be in the form of a powder or a paste.

According to another aspect, the invention provides a method of supplementing the diet of animals with at least one *Cordyceps* species or extract thereof. Preferably, the dietary supplement comprising the extract is mixed with feed and/or drinking liquid and made available to the animal. The animal is preferably an oviparous animal and the method is preferably for producing at least one egg. More preferably, the oviparous animal is poultry. Most preferably, the poultry is fowl. Preferably, the dietary supplement is mixed with the feed and provided to the animal.

According to another aspect, the invention provides drinking liquid supplemented with a dietary supplement comprising at least one extract of at least one *Cordyceps* species.

According to a further aspect, the invention provides an animal feed comprising at least one feed ingredient and at least one extract of at least one *Cordyceps* species. The *Cordyceps* species may be selected from the group consisting of *Cordyceps sinensis*, *Cordyceps militaris*, *Cordyceps gracilis*, *Cordyceps ophioglossoides*, *Cordyceps memorabilis*, *Cordyceps sphecocephala*, *Cordyceps hawkesii*, *Cordyceps martialis*, *Cordyceps sobolifera* and *Cordyceps capitata* . The *Cordyceps* extract may be a powder or a paste. Any of the main feed ingredients may be used for preparing the animal feed, including corn, soybeans, sorghum, oats and barley.

The invention also provides a method of feeding at least one animal with the animal feed described above. The animal is preferably an oviparous animal. More preferably, the animal is poultry. Most preferably, the animal is fowl. In addition, the method of feeding is for producing eggs.

- 5 In another aspect, the invention provides a method of producing at least one egg comprising supplementing the diet of at least one animal with at least one extract of at least one *Cordyceps* species and/or feeding the animal with an animal feed comprising at least one feed ingredient and at least one extract of at least one *Cordyceps* species.
- 10 In another aspect, the invention relates to the use of at least one extract of at least one *Cordyceps* species in the preparation of a dietary supplement for producing at least one egg.

According to another aspect, the invention relates to an egg obtainable according to any of the methods of the invention.

15 Brief description of the figures

- Figure 1 shows the HPLC profiles of (A) whole eggs produced by layers fed animal feed comprising *Cordyceps sinensis* extract (B) eggs produced by control layers (C) cordycepin standard (D) egg yolks from eggs produced by layers fed animal feed comprising *Cordyceps sinensis* extract and (E) egg whites from eggs produced by layers fed animal comprising *Cordyceps sinensis* extract.
- 20

Definitions

Extract includes a product produced by extracting from the original material, an essence from the original material, a concentrate from the original material, an emulsion of the original material, a product produced by drying, grinding or
5 compressing the original material, such as into a powder, a cake, or any product or material derived from the original material, including a paste or dregs.

Oviparous animals refer to animals which lay eggs, with little or no embryonic development within the parent. Oviparous animals comprise many fish, amphibians and reptiles, all birds, the monotremes (mammals which lay eggs),
10 and most insects and arachnids.

Poultry refers to any kind of domesticated bird or bird captive-raised for meat or eggs; ostriches for example are sometimes kept as poultry.

Fowl refers to certain birds often used as food by humans. It includes some poultry such as chickens or turkeys, game birds such as pheasants or partridges,
15 other wildfowl-like guineafowl or peafowl, and waterfowl such as ducks or geese. The birds referred to as "fowl" belong to one of two orders, namely the gamefowl or landfowl (Galliformes) and the waterfowl (Anseriformes).

Animal feed refers to substances for feeding animals, whether solid or liquid or a paste and include compound feed blended from raw materials, fodder and
20 liquids for drinking, such as water.

As used herein, predominantly refers to more than 50%, in particular, more than 60%, more than 70%, more than 80%, more than 90%, or more than 99%.

Detailed description of the invention

For the invention described herein, the at least one extract of at least one *Cordyceps* species for the dietary supplement and/or the animal feed may be derived from naturally occurring *Cordyceps* species. Naturally occurring

5 *Cordyceps* strains grow on the body of a host caterpillar or insect larvae, which becomes mummified by an internal mat of *Cordyceps* mycelium. Eventually, a large, clavate stroma, sometimes as much as 10-15 cm in length grows out from the mummified host and bears an immersed perithecia in its upper part. Wild *Cordyceps* strains are very expensive and can possess high

10 concentrations of heavy metals and also carry microbial contamination. Preferably, the *Cordyceps* extract used is derived from *Cordyceps* species grown in large-scale culture. More preferably, the *Cordyceps* extract used is prepared according to the method disclosed in the Singapore patent application SG 200400686-2, the whole content of which is herein incorporated by

15 reference.

The dietary supplement and/or animal feed comprising the dietary supplement of the invention is beneficial when given to the animal. It will enhance the health and wellbeing of the animal. The *Cordyceps* extract in the dietary supplement and/or animal feed can also stimulate the immune system of poultry, providing

20 improved resistance to diseases including but not limited to fowl cholera, avian influenza including the H5N1 strain, Newcastle disease and/or Marek's disease.

The dietary supplement and/or animal feed may be used both for barn, free range and/or high intensity type rearing of oviparous animals. The dietary supplement and/or animal feed comprising an extract of at least one *Cordyceps*

25 species may be an important advantage in increasing the immunity and reducing susceptibility to diseases, especially in high intensity type rearing of animals.

The present inventors have also surprisingly found that the method described herein results in the production of eggs with reduced cholesterol content. The cholesterol content of chicken eggs is reported to range from 368.20 ± 22.54 to 437.63 ± 24.61 mg cholesterol per 100 g of fresh egg matter (Vorlová *et al.*,
5 2001). The eggs produced by the method of the present invention comprise less than 350 mg cholesterol per 100 g of egg matter. In particular, the eggs produced by the method of the present invention comprise less than 300 mg cholesterol per 100 mg of egg matter. More specifically, the eggs of the present invention comprise 202 to 293 mg cholesterol per 100 g of egg matter.
10 Accordingly, the eggs of the present invention comprise reduced cholesterol content compared to at least one egg not produced by the method of the present invention.

Therefore, according to another aspect, the invention provides an egg comprising less than 350 mg cholesterol per 100 g of egg matter. In particular,
15 the invention provides an egg comprising less than 300 mg cholesterol per 100 g of egg matter.

Further, the present inventors have surprisingly found that the method and use described above results in the transfer of cordycepin into the eggs produced. It has been found that the eggs produced each comprises 0.006 – 0.08 g of
20 cordycepin depending on the amount of dietary supplement provided to the animal. The cordycepin is predominantly in the egg yolk.

Therefore, according to another aspect, the invention provides an egg comprising cordycepin. In particular, the invention provides an egg comprising 0.006 to 0.08 mg cordycepin per 100 of egg matter.

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Cordycepin (3-deoxyadenosine) is a derivative of the nucleoside adenosine produced by *Cordyceps* species. Cordycepin is often used as a biological

marker for *Cordyceps* species. In this instance, the presence of cordycepin in the eggs produced suggests that other active and/or beneficial ingredients of the *Cordyceps* extract are also transferred into the eggs.

The following pharmacological effects of *Cordyceps* have been studied by either animal models and/or human (clinical) trials:

- IMMUNOMODULATION: *Cordyceps sinensis* (CS) can significantly enhance the immunity of the body.
- ANTI-CANCER AND ANTI-MICROBIAL EFFECTS: Associated with immunomodulatory function, CS shows an anti-cancer effect, and was reported to inhibit viral, bacterial and fungal infections.
- ANTIDIABETIC ACTIVITY: Polysaccharide in CS was reported to significantly lower blood glucose levels.
- ENERGY METABOLISM AND PERFORMANCE ENHANCEMENT: CS was reported to stimulate hepatic energy metabolism.
- RESPIRATORY AND PULMONARY FUNCTIONS: CS was reported to relax the bronchial walls as well as having sedative, antitussive, and anti-inflammatory properties, thus it is effective against cough, phlegm, asthma and other respiratory ailments.
- CHOLESTEROL AND LIPID METABOLISM: The report indicated plasma triglyceride, "bad cholesterol" such as LDL-c and vLDL-c, and total cholesterol levels were lowered by CS.
- EFFECTIVE ON RENAL DISORDER: There are indications that CS may be of use in the treatment of Berger's disease (IgA nephropathy).
- EFFECTIVE ON SEXUAL DYSFUNCTIONS AND REPRODUCTIVE DISORDER: Clinical improvement of impotence by taking CS was reported.
- OTHERS: The reports suggested that CS can stimulate erythropoiesis in mouse bone marrow, and inhibits platelet aggregation.

In addition, the compound cordycepin has been extensively studied and has been shown to have anti-tumour and antifungal properties. In one study, it was shown that cordycepin can inhibit nucleolar RNA synthesis in RNA cell (Siev *et al.*, 1969)

- 5 Accordingly, another aspect of the invention provides a method of treating and or prophylaxis of at least one disease comprising administering at least one egg as described herein, an egg extract or egg product from at least one egg as described herein.

10 The invention also relates to the use of at least one egg as described herein, egg extract or egg product from at least one egg as described herein for the preparation of a composition for the treatment and/or prophylaxis at least one disease. The disease includes respiratory disease, kidney disease, renal disease, diabetes, cancer, tumour and/or infectious disease.

15 Having now generally described the invention, the same will be more readily understood through reference to the following examples which are provided by way of illustration, and are not intended to be limiting of the present invention.

Example 1

20 The extract of at least one *Cordyceps* species is preferably obtained from *Cordyceps* cultured according to the method described in published Singapore patent application No. 200400686-2. *Cordyceps* strains with an FIRDI number are available from the Food Industry Research and Development Institute, Taiwan.

Methods are described below of the production of various strains of *Cordyceps*. It should be noted that the parameters given for the conditions of growth such

as fermentation period, temperature, stirring speed, aeration rate, pH range etc, are not necessarily limited to the values as shown but may vary within a reasonable degree as is typically understood by people skilled in the art.

Cultivation of *Cordyceps sinensis* in fermentation device

- 5 A liquid culture medium comprising a carbon source, a nitrogen source, vitamins, minerals and other growth factors from raw materials of glucose, maltose or powder, yeast extract, and food grade chemicals as listed in Table 1 may be used. In order to produce the *Cordyceps*, for example, *C. sinensis*, an isolated strain was first subjected to solid medium culture. The strain *C. sinensis*
 10 APN 4-1 (FIRDI-36421), was grown on and/or kept on yeast malt (YM) slant or plate agar medium (Table 2) or the potato dextrose agar (PDA) medium (Table 3) in test tubes or agar plates at 25°C for 3 days.

For the first seed culture, shake culture making use of Erlenmeyer flasks was used. One litre of the broth medium (Table 4, or Table 5) contained in each
 15 litre conical flask was sterilised at 121°C for 20 minutes and inoculated with 3-6 slant or plate culture of the *C. sinensis* APN 4-1 and were then grown under the following conditions:

- pH 5.5 (adjusted before inoculation)
- 25°C
- 20 • the flasks rotated on shakers at a speed of about 150rpm
- culture period of 48 hr.

For the second seed culture of *C. sinensis*, 75 litre (or bigger) stirred bio-reactors (BioLafitte) each containing 50 litres or more of the fermentation medium (Table 1) were sterilised at 121°C for 30 minutes and then inoculated
 25 with 2 litres of the cells from the 1st seed culture (4% of inoculum). The following growth conditions were maintained:

- pH 5.5
 - 25°C
 - aeration rate of 1:0.2-1:0.9 v/v/m (1500- 2700 l/hr for 50 litres of medium)
- 5
- stirring speed 200rpm
 - cultivation period 48 hr

For the cultivation of mycelia of *C. sinensis*, 800 litre (or a bigger) stirred-tank reactors (for example, BioLafitte), each containing a volume of 600 litres or more of the fermentation medium (Table 1) are inoculated with 50 litres or more of 2nd seeding culture (8% of inoculum). The tanks and the fermentation medium were sterilised at 121°C for 30 minutes before inoculation.

- 10
- pH 5.5
 - 25-29°C
 - stirring speed 200 rpm
- 15
- typical aeration rate of 1:0.5 -1:1 v/v/m (18-36.0 m³/hr for 600 litre medium)
 - cultivation period 48-72 hr

When the mycelium had reached the end of its exponential phase or had multiplied enough for the purpose of production, the bottom valves of the 800 litre bio-reactors were opened, and the mycelium of *C. sinensis* was collected and filtered on vacuum filter or filter pressed; up to 100 kg of mycelium cake with 30% of dry matter was obtained from each bio-reactor. This cake was then dried on a double-cone rotary vacuum dryer or by freeze- drying and a filamentous flour with 27-28% of protein was obtained. The filamentous flour was grounded by a miller to a particle size of 100 -200 mesh. The obtained powder was then packaged into aluminium bags.

Table 1. Liquid Medium

New medium (g/L) The preferred value is indicated in parentheses	
Glucose, 30-50 (40)	
Maltose, 30-60 (40)	
KH ₂ PO ₄ , 0.05-2.0 (1.5)	
MgSO ₄ , 0.05-1.0 (0.3)	
Yeast extract, 15-30 (20)	

Table 2: YM slant/plate agar medium

Ingredient	Content
Yeast extract	3g
Malt extract	3g
Peptone	5g
Dextrose	10g
Agar	20g
Distilled water	Up to 1 litre

5

Table 3. Potato dextrose agar medium

Ingredients	Content
Potato extract	4.0g
Glucose	20.0g
Agar	15.0g
pH	5.6
Distilled water	Up to 1 litre

Table 4. Flask medium-1

Ingredients	Content g/l
Glucose	40.0
Maltose powder	20.0g
Potato dextrose broth	24.0g
Yeast extract	10.0
Water	Up to 1 litre

10

Table 5. Flask medium-2

Ingredients	Content g/l
Glucose	40.0
Maltose powder	40.0

KH ₂ PO ₄	1.5
MgSO ₄ .7H ₂ O	0.3
Yeast extract/peptone	20.0
Water	Up to 1 litre

Cultivation of *Cordyceps gracilis*, *Cordyceps memorabilis*, and *Cordyceps ophioglossoides* in fermentation device

The strains *Cordyceps gracilis* APN 4-2 (FIRDI 32217), *Cordyceps*
5 *memorabilis*, APN 4-3 (FIRDI 32218) and *Cordyceps ophioglossoides*, were
grown and kept on YM slant or plate agar medium (Table 2) at 25°C for 3-5
days. The slant cultures of *C. gracilis*, *C. memorabilis* or *C. ophioglossoides*
were used to inoculate 50 ml of growth medium (described in Table 5, 6) in 300-
ml Erlenmeyer flasks. The mycelia were subjected to shake culture at 25° C for
10 72 hr on a rotating shaker at 150 rpm.

For commercial production of *C. gracilis*, *C. memorabilis*, and *C.*
ophioglossoides, the multi-stage fermentation as described for *Cordyceps*
sinensis above was used.

Cordyceps militaris

15 It was found that this strain would not grow well at a temperature higher than
25°C and also would not grow well on the agar slant/plate. By addition of 0.6%
NaNO₃ or 0.01% Riboflavin (vitamin B2) to the YM agar medium (control, Table
2), the cultivation time was shortened to 3 days (grown on the fortified medium)
from 7 days. *Cordyceps militaris* APN 4-5 (FIRDI 32219) was grown and kept
20 on fortified YM medium at 17-23°C, preferably at 20°C for 3-days before using it
for inoculation of the multi-stage fermentation in Example 3.

The culture medium (Table 1) for the cultivation for all other strains of *Cordyceps*, therefore, differs slightly from that used specifically for *Cordyceps militaris* (Table 6). For all other strains of *Cordyceps*, KH_2PO_4 and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ were used as the mineral source, and for *Cordyceps militaris* NaNO_3 (Table 3) was used instead.

The hot water extraction which increases the amount of active components recoverable from the medicinal fungi. The water used may also be mixed with a pH buffer, adjusting it to about pH 10.

Table 6 Broth Medium for *Cordyceps militaris*

Ingredients	Content, g/l
Glucose	40g
Maltose powder	40g
NaNO_3	2g
Yeast extract/peptone	20g
Water	Up to 1 litre

10

Example 2: Extraction process of *Cordyceps* bio-active ingredients

According to conventional methods known in the art, the active ingredients of *Cordyceps* are extracted for water-soluble and ethanol- soluble compounds. However, these conventional methods of extraction do not include hot water extraction, which is provided in this method and is described below. The absence of a hot water extraction step in conventional methods makes them ineffective in extracting the water-soluble polysaccharides. The hot water may also be buffered at pH 10 for further efficacy.

15

About 1700g of wet *Cordyceps* mycelia were soaked, with gentle agitation, with about 6 litres of hot water (about 90°C) overnight, i.e. about 24hrs or more. The *Cordyceps* were then filtered and the filtrate was then mixed with 2 volumes of 99% ethanol (at 6 litres per volume). The precipitate resulting from the addition
 5 of the ethanol to the filtrate was then collected and washed in 50% ethanol, and subsequently lyophilised, obtaining 2.9g of *Cordyceps* polysaccharides.

The residue was then extracted for active compounds for a further 4 times by using about 4 litres of 80% ethanol solution, with gentle agitation. The ethanol was then evaporated and the resultant concentrate was freeze-dried, obtaining
 10 101g of solids. The use of any other solvent other than methanol, acetone, will be evident to any skilled in the art.

The concentrate is in the form of a powder and may be used as the dietary supplement. The specifications of the powder are shown in Table 7.

Table 7. Product specifications of powdered *Cordyceps* obtained by the method provided
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Product description	Powdered <i>Cordyceps</i> mycelia, produced by submerged cultivation, followed by drying, grinding And sterilization
Product fingerprints Proteins/glycoproteins: Overall:	SDS-PAGE and western blotting Fourier transform infrared (FTIR)spectroscopy
Physical appearance: Colour: Odour: Particle size: Flowing property:	Powder with good flow property Brown or light brown Slightly fermented 100 to 200 mesh, Free-flowing
pH (in 10% solution):	4.5 – 5.5
Moisture %:	Less than 5 %
Active components: Total protein:	27- 28%

Total water soluble polysaccharides:	20% or above
Cordycepic acid (mannitol):	5% or above
Cordycepin (3'-deoxyadenosine):	0.1% or above
Adenosine:	0.15% or above
Ash:	Less than 5%
Heavy metal :	
Arsenic	< 0.01 ppm
Copper	< 15 ppm
Lead	< 0.2 ppm
Mercury	< 0.01 ppm
Microbial limit:	In compliance with the Singapore
Total bacterial count, CFU/g	Medicines Act
Total yeast & mould, CFU/g	< 10 ²
Total E. coli, per gram	< 10 ²
Staphylococcus aureus, per gram	Absent
Salmonella spp., per gram	Absent
	Absent

Example 3: Preparation of Paste Form of *Cordyceps*

Upon completion of the large-scale cultivation methods described in Example 1, the mycelium, instead of being collected and dried, was collected by filtering it through a needle-felt polyester filter medium by gravity or by a controlled vacuum force to make the wet mycelium contain 10-20% of total solids. The wet mycelium was then washed twice by water to remove the residues of the medium.

The yield of the *Cordyceps* paste obtained ranged 300-500 kg per cubic meter of fermentation broth.

The wet mycelium with 10-20% of total solids was subjected to a homogenizing process by either a colloidal mill, or by a homogeniser, or by any type of proper milling machine. A particle size of 0.5-3.0 μm of the wet mycelium was obtained by homogenization. This particle size allowed the wet mycelium to be well

dispersed in the solutions. The obtained *Cordyceps* preparation is indicated as “paste *Cordyceps*”.

The specific characteristics of the obtained “paste *Cordyceps sinensis*” are as shown in Table 8.

- 5 Different characteristics of other species or strains, other than *Cordyceps sinensis* (*C. sinensis*) have also been observed. These characteristics are shown in Table 9.

Table 8: Product specification of *Cordyceps sinensis* in paste form

Product description	<i>Cordyceps sinensis</i> mycelia produced by submerged cultivation, followed by filtration, packaging and retorting at 110 -121°C.
Packaging form	Paste form of products, quantity in 3 kg or customised size, packed in laminated retort pouch.
Product fingerprints Proteins/glycoproteins: Overall:	▪ SDS-PAGE and western blotting ▪ Fourier transform infrared (FTIR) spectroscopy
Physical appearance: Colour: Odour: Property:	Brown or light brown Slightly fermented Paste
pH (in 10% solution):	4.5-5.5
Total solid %:	10-20 %
Components (dry basis): Total protein: Total water soluble polysaccharides: Cordycepic acid (mannitol): Cordycepin (3'-deoxyadenosine): Adenosine:	27 - 28% 20% or above 5% or above 0.1% or above 0.15% or above
Ash:	Less than 5%

Heavy metal:	In compliance with the Singapore Medicines Act
Arsenic	
Copper	< 0.01 ppm
Lead	< 15 ppm
Mercury	< 0.2 ppm
	< 0.01 ppm
Microbial limit:	In compliance with the Singapore Medicines Act
Total bacterial count, CFU/g	
Total yeast & mould, CFU/g	< 10 ²
Total E. coli, per gram	< 10 ²
Staphylococcus aureus, per gram	Absent
Salmonella spp., per gram	Absent
	Absent

Table 9: Characteristics of other Cordyceps strains

Product and cell description	1) <i>C. militaris</i> shows the whitest and finest mycelia visually, while <i>C. sinensis</i> is the most coarse. 2) The paste of <i>C. militaris</i> is more like milk flavour 3) The taste of powdered <i>C. militaris</i> is the sweetest, that of <i>C. ophioglossoides</i> a little sour, and the bitter taste of <i>C. sinensis</i>. 4) Filtered bio-mass of <i>C. militaris</i> mycelia is more compact than that of <i>C. sinensis</i> & <i>C. ophioglossoides</i>. 5) Under microscopy, two spores are contained inside the ascus of <i>C. militaris</i>, as comparison with that of <i>C. sinensis</i>, only one spore (see photos)
FTIR fingerprints	Fingerprints of five species of Cordyceps in Fourier transform infrared (FTIR) spectroscopy are very different, which can be provided if required.
Active compound: Cordycepic acid	Contents of <i>C. militaris</i> & <i>C. ophioglossoides</i> are higher than

	9%, while <i>C. sinensis</i> is 5-7% only.
Active compound: Cordycepin	The content of <i>C. militaris</i> is double or triple higher than that of <i>C. sinensis</i> . But existence of Cordycepin in <i>C. ophioglossoides</i> is not yet confirmed.
Active compound: Adenosine:	Higher in <i>C. militaris</i> and <i>C. ophioglossoides</i> than <i>C. sinensis</i> .
Active compound: Ophiocordin (balanol)	A potent protein kinase inhibitor, is contained in <i>C. ophioglossoides</i> only.

Example 4: Preparation of feed

The animal feed was prepared by mixing 50 g, 125 g or 300g of the *Cordyceps sinensis* powder obtained from Example 2 above per ton of commercial or
5 common laying feed.

Alternatively, the powder may be added to the drinking liquid or the paste may be used instead.

Example 5: Feeding of chickens and properties of eggs produced

8000 Bovans Goldline Brown layers were fed the animal feed comprising
10 *Cordyceps sinensis* prepared as described in Example 4 from 60 weeks to 90 weeks.

During this time, the eggs produced by the layers were sampled and analysed for cordycepin and cholesterol content by HPLC.

Analysis of cordycepin by HPLC

Cordycepin used for standard was obtained from Sigma Chemical Company. For determination of cordycepin in eggs, the soft matter from 8 eggs were combined and the weight was measured. The combination was then
5 homogenised. A 10 g sample of the homogenate was taken and extracted three times with 100 ml of hexane. The extract was collected and sonicated for 1.5 hours at 60 degrees. The sonicate was then filtered with 0.45 micron microsyringe filters before HPLC. The HPLC analysis was performed on a Shimadzu LC20A R1.24 employing 254 nm detection. 5 µl of the filtered
10 sonicate was injected into a 0.45 microsyringe filter µL loop and chromatographed through a C18, 300 x 4.6 mm, 5 µm particle size, 300 Å column (Waters). The mobile phase for elution was acetonitrile-water (V/V 496) with phosphate buffer (Na₂PO₄, 0.1 M, pH 6.8) at a flow rate of 1.0 ml/min. The analysis was performed using the Shimadzu LCsolution software.

15 Analysis of cholesterol content by HPLC

The HPLC analysis of cholesterol content of the eggs was performed, according to standard methodology, by TÜV SÜD, PSB Corporation, Singapore.

Results

Cordycepin was detected in the eggs from layers fed with the animal feed
20 comprising *Cordyceps sinensis* (Figure 1A) and not in the eggs from control layers (Figure 1B). Furthermore, the cordycepin was found predominantly in the egg yoke (Figure 1D). Cordycepin was not detected in the egg white.

Table 10 shows the cordycepin and cholesterol contents of the eggs produced by the layers fed with the animal feed comprising the *Cordyceps sinensis* extract.

Table 10

Addition of CS powder. Gram per ton of chicken layer feed,	Feeding time, weeks	Cordycepin, Gram per 100 gram of egg matter (without shell)	Cholesterol, Milligram per 100 gram of egg matter (without shell)
300 g	8	0.043 – 0.08	202
50 g	12	0.006 – 0.007	237
125 g	10	0.02 - 0.04	293

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The cordycepin levels in the eggs from the layers fed with the feed comprising the *Cordyceps sinensis* extract were measured and were found to be in the range of 0.006-0.08 g per g of whole egg and is dependent on the composition of *Cordyceps* extract in the feed. Similarly, the cholesterol content of the eggs produced were measured and ranged from 202 to 207 mg per 100 g of egg matter. As discussed above, normal eggs have a higher cholesterol content at 368.2 ± 22.54 to 437.63 ± 24.61 mg per 100 g of egg matter (Vorlová *et al.*, 2001).

While there has been described in the foregoing description preferred embodiments of the present invention, it will be understood by those skilled in the technology concerned that many variations or modifications in details of design, construction or operation may be made without departing from the scope of the present invention as claimed.

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References

Siev M., Weinberg, R and Penman, S. (1969) The selective interruption of nucleolar RNA synthesis in HELA cells by cordycepin. *J. Cell Biol.*, vol 41, 510-520.

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Vorlová L., Siegllová E., Karpíšková R. and Kopřiva V. (2001) Cholesterol content in eggs during the laying period. *Acta Vet. Brno*, vol 70, 387-390.

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Claims

- 1 A method of producing at least one egg comprising
 - (i) supplementing the diet of at least one animal with at least one
 Cordyceps species or extract thereof, or
 - 5 (ii) feeding at least one animal with an animal feed comprising at
 least one feed ingredient and at least one *Cordyceps* species or
 extract thereof.
- 10 2. The method according to claim 1, wherein the *Cordyceps* species is
 selected from the group consisting of *Cordyceps sinensis*, *Cordyceps*
 militaris, *Cordyceps gracilis*, *Cordyceps ophioglossoides*, *Cordyceps*
 memorabilis, *Cordyceps sphecocephala*, *Cordyceps hawkesii*,
 Cordyceps martialis, *Cordyceps sobolifera* and *Cordyceps capitata*
- 15 3. The method according to claim 1 or 2, wherein the *Cordyceps* species is
 Cordyceps sinensis.
4. The method according to any one of claims 1 to 3, wherein the egg
 comprises cordycepin.
- 20 5. The method according to claim 4, wherein the egg comprises 0.006-
 0.08 g cordycepin per 100 g of egg matter.
6. The method according to claim 4 or 5, wherein the cordycepin is
25 predominantly in the egg yolk.

7. The method according to any one of claims 1 to 6, wherein the egg comprises reduced cholesterol content compared to at least one egg not produced by the method according to any one of claims 1 to 6.
- 5 8. The method according to any one of claims 1 to 7, wherein the egg comprises less than 350 mg cholesterol per 100 g of egg matter.
9. The method according to any of claims 1 to 8, wherein the egg comprises less than 300 mg cholesterol per 100 g of egg matter.
- 10 10. An egg produced by the method according to any one of claims 1 to 9.
11. An egg comprising cordycepin.
- 15 12. The egg according to claim 11 comprising 0.006-0.008 g cordycepin per 100 g of egg matter.
13. The egg according to claim 11 or 12, wherein the cordycepin is predominantly in the egg yolk.
- 20 14. The egg according to any one of claims 11 to 13, wherein the egg comprises less than 350 mg cholesterol per 100 g of egg matter.
15. The egg according to any of claims 11 to 14, wherein the egg comprises less than 300 mg cholesterol per 100 g of egg matter.
- 25 16. An egg comprising less than 350 mg cholesterol per 100 g of egg matter.

17. An egg comprising less than 300 mg cholesterol per 100 g of egg matter.
18. A method of treating and/or prophylaxis of at least one disease
5 comprising administering
 - (i) at least one egg according to any one of claims 10 to 17, or
 - (ii) an egg extract or egg product from at least one egg according to any one of claims 10 to 17 to a subject.
- 10 19. The method according to claim 18, wherein the disease is selected from the group consisting of respiratory disease, kidney disease, renal disease, diabetes, cancer, tumour and infectious disease.
- 15 20. Use of at least one egg according to any one of claims 10 to 17, or an egg extract or egg product therefrom for the preparation of a composition for the treatment and/or prophylaxis at least one disease.
- 20 21. The use according to claim 20 wherein the disease is selected from the group consisting of respiratory disease, kidney disease, renal disease, diabetes, cancer, tumour and infectious disease.
22. Use of at least one *Cordyceps* species or extract thereof in the preparation of a dietary supplement for producing at least one egg.
- 25 23. The use according to claim 22, wherein the *Cordyceps* species is selected from the group consisting of *Cordyceps sinensis*, *Cordyceps militaris*, *Cordyceps gracilis*, *Cordyceps ophioglossoides*, *Cordyceps*

memorabilis Cordyceps sphecocephala, Cordyceps hawkesii, Cordyceps martialis, Cordyceps sobolifera and Cordyceps capitata.

24. The use according to claim 22 or 23, wherein the *Cordyceps* species is *Cordyceps sinensis*.
25. The use according to any one of claims 22 to 24, wherein the egg comprises cordycepin.
26. The use according to claim 25, wherein the egg comprises 0.006 to 0.08 mg cordycepin per 100 g of egg matter.
27. The use according to any one of claims 25 to 26, wherein the cordycepin is predominantly in the egg yolk.
28. The use according to any of claims 22 to 27, wherein the egg comprises less than 350 mg cholesterol per 100 g of egg matter.
29. The use according to any of claims 22 to 28, wherein the egg comprises less than 300 mg cholesterol per 100 g of egg matter.
30. A dietary supplement comprising an extract of at least one *Cordyceps* species.
31. The dietary supplement according to claim 30, wherein the *Cordyceps* species is selected from the group consisting of *Cordyceps sinensis*, *Cordyceps militaris*, *Cordyceps gracilis*, *Cordyceps ophioglossoides*, *Cordyceps memorabilis*, *Cordyceps sphecocephala*, *Cordyceps hawkesii*, *Cordyceps martialis*, *Cordyceps sobolifera* and *Cordyceps capitata*.

32. The dietary supplement according to claim 30 or 31, wherein the *Cordyceps* species is *Cordyceps sinensis*.

5 33. An animal feed comprising at least one feed ingredient and a dietary supplement according to any one of claims 30 to 32.

34. A method of supplementing the diet of animals, comprising

- 10 (i) providing the dietary supplement according to any one of claims 30 to 32 to at least one animal, and/or
- (ii) feeding at least one animal with an animal feed according to claim 33.

15 35. The method according to claim 34, wherein providing the dietary supplement comprises mixing the dietary supplement with the feed and providing the mixture to the animal.

36. The method according to claim 34 or 35, wherein the animal is an oviparous animal.

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37. The method according to claim 36, wherein the oviparous animal is a poultry.

38. The method according to claim 37, wherein the poultry is a fowl.

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39. The method according to any one of claims 36 to 38, for producing at least one egg.

40. The method according to claim 39, wherein the egg comprises cordycepin.
41. The method according to claim 40, wherein the egg comprises 0.006-0.08 mg cordycepin per 100 g of egg matter.
- 5 42. The method according to claim 40 or 41, wherein the cordycepin is predominantly in the egg yolk.
43. The method according to any one of claims 39 to 42, wherein the egg
10 comprises less than 350 mg cholesterol per 100 g of egg matter.
44. The method according to any one of claims 39 to 43, wherein the egg comprises less than 300 mg cholesterol per 100 g of egg matter.
- 15 45. An animal whose diet is supplemented according to the method of any one of claims 34 to 44.
46. The animal according to claim 45, wherein the animal is oviparous.
- 20 47. The animal according to claim 46, wherein the animal is poultry.
48. The animal according to claim 45 or 46, wherein the animal is fowl.
49. An egg obtainable from the method of any one of claims 39 to 44.