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Welcome to the ninth issue of Mycology News, a newsletter for healthcare professionals dedicated to the dissemination of information on the clinical use of mushroom nutrition.

In this edition, we look at the role of *Cordyceps sinensis* supplementation in liver disease and of mushroom nutrition in detoxificiation. Dr. Girao Bastos (Vet) outlines the clinical rational for the use of *Coriolus versicolor* supplementation as immunonutrition in small pets and we have an update on the use of mushrooms in sports nutrition.

Hepato-protective Effect of Cordyceps sinensis in Cases of Liver Damage

Cordyceps sinensis is most commonly used to support adrenal, lung and immune function but there is also a growing body of evidence supporting its important hepato-protective properties [1].

In a study with 33 chronic hepatitis B patients, cultured *Cordyceps sinensis* mycelia was reported to improve liver function, raise plasma albumin and adjust protein metabolism (Zhou et al, 1990)[2]. In another study 45 patients were treated for post-hepatic cirrhosis with *Cordyceps sinensis* and an extract of *semen Persicae*, with improvement in NK cell function, T-cell ratio and numbers, immunoglobulin levels, serum complement levels, and liver function compared to a control group (Zhu and Lin, 1992).[3] It has also been reported to be of benefit in the treatment of chronic obstructive hepatic diseases (Chang & But 1986). [4]

In 2003, two important studies were conducted at the Clinical College of Chongqing University of Medical Sciences in China on the impact of *Cordyceps sinensis* supplementation in hepatic care. The first study looked at the effect and mechanism of action of *Cordyceps sinensis* on the activity of hepatic insulinase in CC1[4] induced liver cirrhosis in rats. The conclusion of the study was that *Cordyceps sinensis* supplementation could decrease hepatocyte damage and inhibit hepatic fibrogenesis.[5]

The second study looked at the inhibitive effect of *Cordyceps sinensis* on CCl[4] and ethanol-induced hepatic fibrogenesis in rats. Biochemical, radioimmunological, immunohistochemical and molecular biological examinations were used to determine the change of ALT, AST, HA, LN content in serum and TGFbeta[1], PDGF, collagen I and III expression in tissue at either protein or mRNA level or both.

The study concluded that *Cordyceps sinensis* supplementation could inhibit hepatic fibrogenesis derived from chronic liver injury, retard the development of cirrhosis, and notably improve liver function. A possible mechanism involves the inhibition of TGF beta[1] expression and thereby the down regulation of PDGF expression, thus preventing HSC activation and the deposition of procollagen I and III [6].

Taken together, these studies show a clear hepato-protective effect of *Cordyceps sinensis* and confirm its potential benefit in cases of liver damage.

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Cordyceps sinensis Supplementation as Immunonutrition in Alcohol Induced Liver Steatosis-II

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Introduction:

Immune deregulation is a component of the pathogenesis of liver disease. Conversely drugs that affect immune function may be beneficial in treating these diseases. However, in the absence of cost-effective drugs, there is a set of nutrition based approaches that have been used to improve the immune function in patients with liver disease.

For instance, in alcohol-induced liver disease there is an overproduction of pro-inflammatory cytokines such as TNF by peripheral blood monocytes and resident hepatic Kupffer cells [1].

Dietary supplementation by polyamines or by S-adenosyl-methionine (SAMe), has been termed "immunonutrition", and has been postulated to improve gut or systemic defenses to infection and injury[2]. Patients with alcohol-induced liver diseases have decreased SAMe levels, which may predispose them to mitochondrial glutathione depletion and dysfunction. Intravenous SAMe administration to individuals with alcohol-induced liver disease was reported to improve methionine clearance and liver function in four patients to date[3].

Mushroom nutrition in the form of *Cordyceps sinensis* supplementation is also gaining ground as immunonutrition for patients with alcohol-induced liver steatosis and this paper presents information on the use of *Cordyceps sinensis* supplementation in fourteen[14] patients with alcohol-induced liver steatosis.

Background on Alcohol Induced Liver Steatosis:

Alcohol induced liver steatosis is macrovascular steatosis rather than microvascular steatosis. (i.e. Reye´s Syndrome)[4]. Equally as important as hepatocyte injury from prolonged alcohol use is the remarkable predilection for collagen formation seen in alcoholic liver disease. Transformation of fat-storing parasinusoidal Ito cells into myofibroblasts with chronic alcohol exposure is an important line of current research.[5]

In 1985, Tsukamoto et al. reported a rat model of intragastric ethanol infusion capable of producing hepatocyte necrosis, inflammation, and fibrosis in addition to steatosis.[6]

In most acute hepatocellular disorders, the alanine aminotransferase (ALT or serum glutamic pyruvic transaminase (SGOPT) level is higher than or equal to the aspartate aminotransferase (AST or serum glutamic oxaloacetic transaminase (SGOT)[7]. Gamma glutamyl transpeptides may be disproportionately elevated in the sera of patients who abuse alcohol, even in the absence of obvious liver cell injury.[8]

The presence of a disproportionately elevated serum gamma glutamyl transpetidase (GGPT) and an AST/ALT ratio greater than 2:1 should alert the physician to the possibility of alcoholic liver disease[9]. Patients with alcoholic fatty liver have virtually normal hepatic tests except for mild transient increases in serum aspartate transaminase (AST) and gamma glutamyl transpeptidase (GGTP). The ratio of serum mitochondrial AST to total AST is often abnormally increased. Raised serum urate, lactate, or triglycerides may be present as clues to alcoholic causes of liver enlargement. [10]

Background on Cordyceps sinensis:

The pharmacologically active components of *Cordyceps sinensis* are still unresolved. Cordycepin and cordycepic acid have been proposed as important active constituents **(Pegler et al, 1994; Zhu et al, 1998)**, and it is now believed that cordycepic acid is, in fact, d-mannitol, and that cordycepin is 3'-deoxyadenosine **(Zhu et al, 1998a)**.

It seems unlikely however that either, or both, of these simple compounds could be responsible for the varied and complex reported physiological actions of *Cordyceps sinensis*. **(Kiho et al 1986, 1993, 1996, 1999)** have conducted a series of experiments in which they have extracted a number of polysaccharides from *Cordyceps sinensis*, which they have been reported to exhibit hypoglycaemic effects in normal and diabetic mice.

In 2003, based on rat studies, researchers at the Chongqing University of Medical Sciences concluded that *Cordyceps sinensis* could inhibit hepatic fibrogenesis derived from chronic liver injury, retard the development of cirrhosis, and notably improve liver function. In 2004, other researchers at Chongqing University of Medical Sciences concluded that *Cordyceps sinensis* may decrease hepatocyte damage by CC14, and inhibit hepatic fibrogenesis[12].

Safety and Toxicity of Cordyceps sinensis

There is abundant animal based literature to show that *Cordyceps sinensis* is a very safe product **(Huang et al, 1987; Lin et al, 1999, Wang & Zhao, 1987; Zhu et al, 1998b, Manabe et al, 2000).**

There have been very few reports of adverse reactions to *Cordyceps sinensis* in humans and it is freely available to the

general public as a dietary supplement. The recommended daily dose of *Cordyceps sinensis* for ingestion in adults is most commonly 1 g, 3 times a day **(China-guide, 2000)**.

There have been only two reports of adverse effects of supplementation in humans **(Shao et al, 1990; Xu, 1992)**, which were reports of nausea, dry mouth and stomach discomfort.

Aim of Study

There is no known treatment for alcoholic fatty liver beyond alcohol withdrawal and a nutritious diet. The key clinical objective was to assess the efficacy of non-fractionalized *Cordyceps sinensis* supplementation (3 grams per day), over 90 days, in reducing the following liver enzyme levels in fourteen (14) patients with alcohol induced liver steatosis over 20 days and 90 days.

1) Aspartate Aminotransferase (AST) or Serum Glutamic Oxaloacetic Transaminase (SGOT)

2) Alanine Aminotransferase (ALT) or Serum Glutamic Pyruvic Transaminase (SGPT)

3) Gamma Glutamyl Transpetidase (GGT)

4) Phosphatase (alkaline) (PA)

The normal ranges for these enzyme levels should be:

AST-0-35 IU/L

ALT-5-35 IU/L

GGT-very variable

Supplementation Schedule:

The supplementation level used was 3 grams of *Cordyceps sinensis* (non-extracted) taken in tablet form (500 mg) 3 tablets per day (1.5 gram) in the morning (30 minutes prior to breakfast) and 3 tablets per day (1.5 grams) in the evening (30 minutes prior to dinner).
The supplementation period was 90 days, with measurements taken at day 0, day 20 and day 90.*

General Observations on Sample Size

Of the fourteen (14) patients, three (3) were women (average age 45 years) and eleven (11) were men (average age 41 years).

Effect of Aqueous Extract of Cordyceps sinensis on HepG2 Liver Cancer Cells

by Dr. Chris Newton (PhD), Cytogenex Ltd, 121 Westbourne Avenue, Kingston-upon-Hull, East Yorkshire. HU5 3HU. Dr. Newton is a Senior Fellow, University of Hull School of Medicine and Senior Visiting Fellow, Max-Planck Institute, Munich.

In 2003 Cytogenex Laboratories was requested by Mycology Research Laboratories Ltd. to determine whether *Cordyceps sinensis* (biomass form) had any significant toxicity when tested with a standard toxicity screen. A system was developed by Cytogenex Laboratories based on the liver cell line, HepG2. Five concentrations of *Cordyceps sinensis* aqueous extract were prepared (aliquots of 50, 25,12.5, 6 and 3µl; 10mg/ml - 0.6mg/ml final concentrations) and added to the HepG2 cell line. The cells were monitored daily over a 5 day period for signs of cell death.

No obvious response was apparent from microscopic observations in the initial 48 hours. Over the subsequent 72 hour period, it was clear that the cells in the presence of higher doses of Cordyceps extracts (25 and 50 µl) grew more slowly.

In a separate experiment, HepG2 cell lines were seeded into cultures plates and an aliquot (50µl) of *Cordyceps sinensis* was added.

After 48 hours, a range of H_2O_2 doses (200 to 600 µM) was added to the HepG2 cell lines in the presence of *Cordyceps sinensis* extract. The results indicated that the *Cordyceps sinensis* extract partly blocked the H_2O_2-induced fall in viable cell numbers at the higher doses of oxidant.

Therefore, by the definition of cytotoxicity given by Cytogenex, the aqueous extract of *Cordyceps sinensis* was apparently devoid of toxicity in this and most probably, other cell systems.

For a complete report on this experiment, please contact Cytogenex at info@cytogenex.com

In addition to these studies to determine the safety of fungal biomass extracts, Cytogenex can offer a certification service where the identity of the product can be matched against a sample previously verified as authentic. This service uses a DNA fingerprinting technique. For further information with regard to services provided to address aspects of nutraceutical product quality and safety, please visit the web site at: www.cytogenex.com

I) Observations on Changes in Asparate Aminotransferase (AST) or Serum Glutamic Oxaloacetic Transaminase (SGOT) Levels over 20 days and 90 days

The following Table I provides an overview of the decrease in AST (SGOT) levels with Cordyceps sinensis supplementation (3.0 grams per day) over 90 days.

TABLE I

Day	0	20	90	Day 0 vs Day 20	Day 0 vs Day 90
				%	%
AST **(A)**	336	97	35	-71%	-90%
AST **(B)**	118	15	19	-87%	-84%
AST **(C)**	98	95	15	-3%	-85%
AST **(D)**	229	348	35	52%	-85%
AST **(E)**	448	224	75	-50%	-83%
AST **(F)**	45	37	17	-18%	-62%
AST **(G)**	448	228	54	-49%	-88%
AST **(H)**	340	28	27	-92%	-92%
AST **(I)**	45	37	35	-18%	-22%
AST **(J)**	225	98	54	-56%	-76%
AST **(K)**	58	30	29	-48%	-50%
AST **(L)**	548	34	38	-94%	-93%
AST **(M)**	45	34	37	-24%	-18%
AST **(N)**	98	47	35	-52%	-64%
				-611%	-992%
			Av	-43,6%	-70,8%

After 20 days of Cordyceps sinensis supplementation at 3.0 grams per day, the average reduction in AST (SGOT) was 43,6%. After 90 days of supplementation the average reduction was 70,8%.

With ANOVA (analysis of variance) statistical analysis the difference for AST (SGOT) between the group at 0-20 days and at 0-90 days was $p<0.001$.

Using a one-way ANOVA for 3 correlated samples (zero time, 20 days and 90 days) gave an AST (SGOT), ANOV p value=0.00023 (Turkey HSD Test between 0 and 20 days, $p<0.05$, between 0 and 90 days, $p<0.01$.

II) Observations on Changes in Alanine Aminotransferase (ALT) or Serum Glutamic Pyruvic Transaminase (SGPT) Levels over 20 days and 90 days

The following Table II provides an overview of the decrease in ALT (SGPT) levels with *Cordyceps sinensis* supplementation (3.0 grams per day) over 90 days.

TABLE II

Day	0	20	90	Day 0 vs Day 20	Day 0 vs Day 90
				% _	%
ALT **(A)**	98	74	15	-24%	-85%
ALT **(B)**	224	48	40	-79%	-82%
ALT **(C)**	48	40	39	-17%	-19%
ALT **(D)**	324	58	43	-82%	-87%
ALT **(E)**	86	74	37	-14%	-57%
ALT **(F)**	86	30	28	-65%	-67%
ALT **(G)**	327	98	45	-70%	-86%
ALT **(H)**	348	95	47	-73%	-86%
ALT **(I)**	42	40	40	-5%	-5%
ALT **(J)**	328	35	37	-89%	-89%
ALT **(K)**	35	30	30	-14%	-14%
ALT **(L)**	344	48	45	-86%	-87%
ALT **(M)**	48	30	29	-38%	-40%
ALT **(N)**	228	75	43	-67%	-81%
				-723%	-885%
			Av	-51,6%	-63,2%

After 20 days of *Cordyceps sinensis* supplementation at 3.0 grams per day the average reduction in ALT (SGPT) levels was 51.6%. After 90 days of supplementation the average reduction was 63.2%.

Using a one-way ANOVA for 3 correlated samples (zero time, 20 days and 90 days) gave an ALT (SGPT), ANOV p value <0.0001 (Turkey HSD Test between 0 and 20 days, $p<0.01$, between 0 and 90 days, $p<0.01$.

(III) Observations on Gamma Glutamyl Transpetidase (GGT) levels over 20 days and 90 days

The following Table III provides an overview of the decrease in Gamma Glutamyl Transpetidase (GGT) levels with *Cordyceps sinensis* supplementation (3.0 grams per day) over 90 days

TABLE III

Day	0	20	90	Day 0 vs Day 20	Day 0 vs Day 90
				%	**%**
GGT **(A)**	534	228	75	-57%	-86%
GGT **(B)**	98	96	15	-2%	-85%
GGT **(C)**	228	224	197	-2%	-14%
GGT **(D)**	1124	445	97	-60%	-91%
GGT **(E)**	85	94	35	11%	-59%
GGT **(F)**	48	40	39	-17%	-19%
GGT **(G)**	127	95	35	-25%	-72%
GGT **(H)**	54	35	40	-35%	-26%
GGT **(I)**	37	35	34	-5%	-8%
GGT **(J)**	548	223	48	-59%	-91%
GGT **(K)**	224	48	34	-79%	-85%
GGT **(L)**	1144	527	35	-54%	-97%
GGT **(M)**	224	37	36	-83%	-84%
GGT **(N)**	228	75	43	-67%	-81%
				-536%	-898%
			Av	-38,3%	-64,1%

After 20 days of *Cordyceps sinensis* supplementation (3.0 grams per day) the average reduction in GGT was 38.3%. After 90 days supplementation the average reduction was 64.1%.

Using a one-way ANOVA for 3 correlated samples (zero time, 20 days and 90 days) gave a GGT, ANOVA p value=0.0018 (Turkey HSD Test between 0 and 20 days, $p<0.05$, between 0 and 90 days, $p<0.01$.

(IV) Observations on Phosphatase Alkaline (PA) levels over 20 days and 90 days

The following Table IV provides an overview of the decrease in Phosphatase alkaline (PA) levels with *Cordyceps sinensis* supplementation (3.0 grams per day) over 90 days.

TABLE IV

Day	0	20	90	Day 0 vs Day 20	Day 0 vs Day 90
				%	%
PA **(A)**	115	98	97	-15%	-16%
PA **(B)**	224	111	98	-50%	-56%
PA **(C)**	117	115	120	-2%	3%
PA **(D)**	75	72	74	-4%	-1%
PA **(E)**	117	117	95	0%	-19%
PA **(F)**	98	100	102	2%	4%
PA **(G)**	154	117	110	-24%	-29%
PA **(H)**	115	117	111	2%	-3%
PA **(I)**	104	99	98	-5%	-6%
PA **(J)**	120	117	115	-3%	-4%
PA **(K)**	115	117	109	2%	-5%
PA **(L)**	128	115	117	-10%	-9%
PA **(M)**	113	117	115	4%	2%
PA **(N)**	117	115	117	-2%	0%
				-105%	-139%
			Av	17,5%	-23,2%

After 20 days of *Cordyceps sinensis* supplementation (3.0 grams per day) the average reduction in Phosphatase alkaline (PA) was 17,5%. After 90 days supplementation the average reduction was 23.2%.

There was no statistically significant difference in Alkaline Phosphate levels between groups.

Results:

When provided Cordyceps sinensis supplementation of 3.0 grams per day, the change in liver enzyme levels in fourteen (14) patients with alcohol induced liver staetosis over 20 days and 90 days was significant.

The summary results are provided in Table V:

Summary	Day 0 vs Day 20 % 20 days	Day 0 vs Day 90 % 90 days	% ANOVA	Turkey HSD Test 0 and 20 days	Turkey HSD Test 0 and 90 days
1 AST (SGOT)	-43,6%	-70,8%	p=0.00023	p<0.05	p<0.01
2 ALT(SGPT)	-51,6%	-63,2%	p=0.0001	p<0.01	p<0.01
3 GGT	-38,3%	-64,1%	p=0.0018	p<0.05	p<0.01

Conclusion

When compared to the normal procedure of "abstinence", Cordyceps supplementation as immunonutrition demonstrates a significant reduction in liver enzyme levels within 20 days, while under "abstinence", a reduction in liver enzymes is usually detected only after 45 days. Given that *Cordyceps sinensis* supplementation costs between € 65.00 to € 70,00 (£43.47-46.81, $79.81-85.95) per month, the daily cost of immunutrition would be € 2.17 to € 2.35 (£1.45-1.56, $2.66- 2.87) per day.

Cordyceps sinensis supplementation as immunonutrition in patients with alcohol induced liver steatosis offers the clinician a cost effective option for such cases.

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Notes:

1) The Cordyceps sinensis was supplied by Mycology Research Laboratories Ltd. (http://www.mycologyresearch.com)

2) Wild Cordyceps sinensis is rare, and artificial cultivation now dominates production. This confers the advantage that the Cordyceps is not contaminated by bacteria, other fungi and heavy metals. The Cordyceps sinensis used was a biomass powder containing the mycelia and primordial of Cordyceps sinensis cultivated on a sterile substrate. The biomass was then manufactured into 500 mg tablets in the United Kingdom (to pharmaceutical GMP standards).

3) The enzyme content of each 500 mg tablet (with additives 325 mg) of has the following in vitro profile in the absence of protelytic enzymes:

Cytochrome P-450	0.25 nmoles
Cytochrome P-450 reductase	4.14 mU
Superoxide dismutase (SOND) activity	77.14 U
Peroxidase activity :	57.2 mU/tablet
Protease activity	5.6 U/tablet

4) The enzyme analysis is performed by Associate Professor Dr. Amin Karmali at the Biotechnology Section, Instituto Superior de Engenharia de Lisboa, Portugal (Fax:21-831-7267 / akarmali@isel.ipl.pt))

Detoxification - The Role of Mushroom Nutrition

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A number of pathological damages including carcinogenesis and cellular degeneration related to aging are due to reactive oxygen species (ROS), or superoxide radicals. These reactive oxygen species are produced by sunlight, ultraviolet radiation, chemical reactions, as well as by metabolic processes, and are toxic to living cells since they oxidize and degrade important biological macromolecules such as lipids and proteins (1).

Health maintenance and the avoidance of chronic degenerative conditions therefore depends to a large extent on the body's ability to neutralise, in other words detoxify, such ROS.

Central to the body's battle against ROS are a number of enzyme systems, prominent among which is Superoxide dismutase (SOD), which catalyses the destruction of superoxide radicals and hence protects oxygen–metabolizing cells from the harmful effect of these free radicals. Several research workers have shown that SOD is involved in diseases such as Parkinson´s disease, cancer and anemia (1,2).

Another important enzyme system is cytochrome "P-450" which is located in the endoplasmic reticulum and plays an important role in metabolism and detoxification of endogenous substances (3). In addition, enzyme therapy has been shown to play an important role in several clinical conditions including cancer, malignant lymphomas and cardiovascular disorders (4,5).

Mushrooms have been known to possess medicinal properties for thousands of years and higher basidiomycete mushrooms have been used in clinical nutrition for their anti-tumour, immune modulating, cardiovascular and anti-microbial effects (6). As well as other complex substances of therapeutic interest, such as protein-bound polysacharide complexes (i.e PSK, PSP and Lentinan) and secondary metabolites (i.e terpenes, alkaloids and lactones) we are now finding that mushrooms are rich sources of many enzymes.

Several mushrooms have been shown to contain substances which mimic SOD activity (7) and the "P450" cytochrome enzyme system has also been found in some higher basidiomycete fungi. Other enzymes present in clinically used mushrooms include laccase, glucose oxidase and peroxidase (8).

It is likely that the potent enzymatic and ROS detoxifying properties of mushrooms are in large part due to the harsh environments colonized by mushroom mycelia with high concentrations of free radicals that the mushrooms have to protect themselves against.

In this connection it is worth noting that these enzymes are found almost exclusively in the mushroom mycelia and hence preparations derived from the fruiting bodies of mushrooms are likely to have far lower levels of enzymatic activity than those derived from mushroom mycelia.

In the present work, we investigated the levels of SOD, cytochrome "P450", cytochrome "P450" reductase (NADPH dependent) and secondary thrombin inhibiting metabolites in the following mushrooms: *Coriolus versicolor, Cordyceps sinensis, Ganoderma lucidium* (Reishi) and *Grifola frondosa* (Maitake).

There are a number of secondary metabolites in mushrooms which play an important role as thrombin inhibitors (10) and since thrombin is an important protease of the coagulation system it is a suitable target for inhibition of blood coagulation, which is desirable in combating many age related conditions.

In order to simulate the human intestinal tract we treated the mushrooms with the following proteolytic enzymes:

1. Pepsin (500IU/g biomass) at pH2 for 30 min. at 37°C in an incubator with orbital shaking

2. Trypsin (500IU/g biomass) at pH 7.6 for 30 min. at 37°C in an incubator with orbital shaking.

The analysis of SOD, cytochrome "P450", cytochrome "P450" reductase (NADPH dependent) and secondary thrombin inhibiting metabolites in *Coriolus versicolor, Cordyceps sinensis, Ganoderma lucidium* (Reishi) and *Grifola frondosa* (Maitake) produced the following results:

Table 1- In the absence of Proteolytic Enzymes

Enzymes and secondary metabolites	Maitake (Grifola frondosa)	Reishi (Ganoderma lucidum)	Coriolus versicolor	Cordyceps sinensis
Analysis Per Tablet (500 mg)*				
1 Superoxide dismutase (SOD) activity	70.2U	50.4U	77.1U	77.1U
2 Cytochrome "P450"	0.60 nmoles	0.66 nmoles	0.51 nmoles	0.25 nmoles
3 Cytochrome "P450" reductase	7.14 mU	7.05 mU	11.9mU	4.14mU
4 Secondary metabolites (Thrombin inhibitors)	49%	4.4%	59%	56%

Table 2 - In the Presence of Pepsin

Enzymes and secondary metabolites	Maitake (Grifola frondosa)	Reishi (Ganoderma lucidum)	Coriolus versicolor	Cordyceps sinensis
Analysis Per Tablet (500 mg)*				
1 Superoxide dismutase (SOD) activity	58.7U	41.3U	61.2U	49.5U
2 Cytochrome "P450"	0.48 nmoles	0.53 nmoles	0.49 nmoles	0.24 nmoles
3 Cytochrome "P450" reductase	6.06mU	5.92mU	9.52mU	3.80mU
4 Secondary metabolites (Thrombin inhibitors)	46.5%	3.7%	54.2%	50.9%

Table 3- In the Presence of Trypsin

Enzymes and secondary metabolites	Maitake (Grifola frondosa)	Reishi (Ganoderm frondosa)	Coriolus versicolor	Cordyceps sinensis
Analysis Per Tablet (500 mg)*				
1 Superoxide dismutase (SOD) activity	69.5U	51.4U	68.5U	90.6U
2 Cytochrome "P450"	0.58 nmoles	0.63 nmoles	0.52 nmoles	0.24 nmoles
3 Cytochrome "P450" reductase	7.03mU	6.98mU	11.1mU	4.02mU
4 Secondary metabolites (Thrombin inhibitors)	46%	3.7%	52%	57%

The data presented in these tables reveal that simulation of the intestinal tract with pepsin and trypsin decreased the enzyme and secondary metabolite levels by 15-20%.

Conclusions:

Mushrooms contain several important enzymes involved in detoxification process (i.e cytochrome "P450") and destruction of superoxide free radicals (i.e SOD activity) as well as secondary metabolites which act as thrombin inhibitors.

Further research is required to study the effect of mushroom nutrition on the levels of some key proteins and enzymes in vivo which are involved in several clinical conditions including cardiovascular diesases, cancer, HIV and neurological disorders.

* Mushroom samples (in tablet form) were composed of the mycelium and primordia of the respective mushrooms and were provided by Mycology Research Laboratories Ltd. www.mycologyresearch.com

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Mushroom Nutrition in Sports Nutrition

The modern use of mushroom nutrition as adjunct nutrition for sportsmen was pioneered by the Chinese athletes of the early nineties who included Cordyceps sinensis as part of their supplementation regime in pursuit of record breaking performances. Its use in this area draws upon its centuries old usage in traditional Chinese medicine for fatigue and lung weakness.

Perhaps the ultimate test for athletes today is the triathlon, where athletes compete over one of three distances: Olympic, 1/2 Ironman and Ironman.

	Olympic	1/2 Ironman	Ironman
Swim	1.5 km	1.2 miles	2.4 miles
Bike	40 km	56 miles	112 miles
Run	10 km	13.1 miles	26.2 miles

Since 1998 Mycology Research Laboratories Ltd. has been sponsoring Chad Hawker, one of the most accomplished triathletes in America who has maintained a top 10 US and Top 50 International ranking since 2001.

A key issue for high performance ironman athletes is chronic fatigue caused by the high volume of rigorous training (15 miles of swimming, 400 miles of biking, 60 miles of running every week). In order to minimize the potential for chronic fatigue due to overtraining, Chad makes use of mushroom nutrition's immuno-modulatory properties in his supplementation schedule. He uses Coriolus-MRL in his pre-event programme and Cordyceps-MRL in his post-event recovery programme and has reported a lower incidence of illness, more rapid recovery from colds, faster post-event recovery and improvement in

**Coriolus-MRL	1 Tablet=500 mg**	
Pre-Event Supplementation Program	14 days before event	6 tablets per day*
Maintenance Training Program		3 tablets per day**
**Cordyceps-MRL	1 Tablet=500 mg**	
Recovery Supplementation Program	14 days after event	6 tablets per day*
Maintenance Training Program		3 tablets per day**

*** 3 tablets (30 minutes prior to breakfast) / 3 tablets (30 minutes prior to dinner) **3 tablets (30 minutes prior to breakfast)**

For information on Chad Hawker's accomplishments please see www.HawkForLife.com, or you can email Chad at: fasthawk@earthlink.net

We are pleased to report that on May 31st, 2004 Chad won the Keauhou Kona 1/2 Ironman for the 6th consecutive year (1999-2004)

Mushroom Nutrition as Immunonutrition in Cats and Dogs – Three Case Studies by Dr. Girao Bastos

Dr. Girao Bastos-Laboratório Nacional de Investigação Veterinária (Fax: 21-484-9629)

Coriolus versicolor supplementation may provide veterinarians with another tool when faced with palliative care decisions in small pets. Based on my experience with *Coriolus versicolor* supplementation in small animals (dogs and cats), the supplementation improves the immune state of small animals with viral conditions as well as the quality of life in palliative care.

The potential to modulate the activity of the immune system by interventions with specific nutrients is termed immunonutrition. This concept may be applied to any situation in which an altered supply of nutrients is used to modify inflammatory or immune responses.(1).

However, *Coriolus versicolor* supplementation is not a substitute for any existing medical procedure or medical product. *Coriolus versicolor* supplementation should be considered as a complementary immunonutrition product to support an animal´s immune system. The following article outlines clinical experience with *Coriolus versicolor* supplementation in cats and dogs.

Clinical History on Coriolus versicolor

In Japan, an extract of the fungus *Coriolus versicolor*, Krestin, (PSK), is used as the basis of immunotherapy in cancer patients. In cancer care, Krestin (PSK) is provided to patients undergoing either radiotherapy, chemotherapy or surgery to improve the patient´s immune system(2).

Two proteoglycans from *Coriolus versicolor*-PSK (Polysaccharide-K) and PSP (Polysaccharide-peptide) have demonstrated the most promise. In Japanese human trials since 1970, PSK significantly extended survival at five years or beyond in cancers of the stomach, colon-rectum, esophagus, nasopharynx, and lung (non-small cell types), and in a HLA B40-positive breast cancer subset.(3)

Both PSK and PSP boosted immune cell production, ameliorated chemotherapy symptoms, and enhanced tumor infiltration by dendritic and cytotoxic T-cells.

Their extremely high tolerability, proven benefits to survival and quality of life, and compatibility with chemotherapy and radiation therapy makes them well suited for cancer management regimens.(4).

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(2) The Use of Mushroom Glucans and Proteoglycans in Cancer Treatment- Dr. Parris Kidd (Ph.D)-Alternative Medicine Journal 2000;5(1);p 4-27.

(3) Ibid, p 4-27

(4) Ibid, p4-27

Case Studies with Coriolus versicolor Supplementation in Small Pets*

Case Study 1: Subject: Cat with Feline Leukemia (FELV)

Diagnosis:

In May of 2001, a three (3) year old cat was found to be tired, sad, lacked appetite, had lost weight and was seriously dehydrated. The cat had tested positive for FELV and negative for FIV in March of 2001 (in another laboratory). FELV is known as cat leukemia and the A decision was made to assist the immune system with *Coriolus versicolor* supplementation

May 1st, 2001 *Coriolus versicolor* supplementation initiated with the following schedule:

Days 1-15 - 2 tablets per day (crush tablet (500 mg) and mix with meals)

Days 15-90 - 1 tablet per day (crush tablet (500 mg) and mix with meals)

Observation 1 1 - July 7th of 2001
Rectal temperature 37.9°C.

Cat in good state and able to move around with ease.

Observation 2 - August 19th of 2001 -
Rectal temperature 38.5°C

Cat is in a good state of health with much more energy and better appetite. Decision made to continue supplementation at 1 tablet per day.

Observation 3 - October 26th of 2001

For a period of 15 days in October the cat did not take supplementation and lost 1 kilo with a noted decrease in physical activity. *Coriolus versicolor* supplementation reinitiated and maintained at 1 tablet (500 mg) per day.

Observation 4 - January 2nd, 2002

Coriolus versicolor supplementation reduced to 1 tablet (500 mg) every three (3) days.

Observation 5 - August of 2004 `

Cat remains on *Coriolus versicolor* supplementation 1 tablet (500 mg) every three (3) days and is still active.

Comments: *Coriolus versicolor* supplementation is not able to eliminate the FEVL virus but it does provide a method to manage the virus by supporting the cat´s immune system. The cat continues with a maintenance Coriolus supplementation of 1 tablet every three (3) days to provide immune support. This approach allows the immune system to be fortified against environmental changes that may reduce the cat´s immune system; while maintaining the FEVL viral load at a "manageable level".

Case Study 2 Subject: Cocker Spaniel with abdominal tumor (palliative care)

Diagnosis:

On July 26th 2001 a ten (10) year old cocker spaniel was diagnosed with an abdominal tumor after undergoing an X-ray test. Rectal temperature 38.5°C.

Prognosis was poor but rather than "putting down" the patient, a decision was made to assist the immune system with the use of *Coriolus versicolor* supplementation.

On August 1st, 2001 with rectal temperature of 38.3°C, *Coriolus versicolor* supplementation was initiated with the following schedule:

Days 1-15 - 2 tablets per day (crush tablet (500 mg) and mix with meals)

Days 15 onwards - 1 tablet per day (crush tablet (500 mg) and mix with meals)

Observation 1 - September 29th 2001 - Rectal temperature 38.3°C.

Cocker maintains health status with no deterioration in quality of life.

Observation 2 - December 8th 2001 - Rectal temperature 38.4°C

Cocker maintains health status with no deterioration in quality of life.

Observation 3 - March 25th 2002

Cocker has more energy and is playful with the second dog in the home and with his toys.

Observation 4 - May 5th of 2002

Cocker dies in sleep from cardiac arrest.

Comments: In terms of palliative care, *Coriolus versicolor* supplementation provided an option to improve the quality of life for the cocker spaniel by improving the energy level. Although there was no change in the tumor size during supplementation, *Coriolus versicolor* supplementation may still offer an alternative to "putting down" both dogs and cats in the palliative stages of cancer care.

Case Study 3 Subject: Cat with Fibrosarcoma in the hip area and Haemabartonella felis infection

Diagnosis:

On September 2002, an eight (8) year old cat was found to have severe discoloration on the abdominal portion of the body and to be suffering from anemia. A pathological examination was conducted to determine the possibility of fibrosarcoma. The test found that the discoloured areas were composed of a great proliferation of interrelated fusiform cells with oval shaped nucleui in elongated form. Furthermore, the cells were irregularly shaped. It was observed that these cells were undergoing abundant mitosis.

Furthermore the observation that the citoplasm had a fibrial form, and that some cells contained occasional multinucleation, confirmed the existence of fibrosarcoma.

However, to explain the anemia, a diagnostic test was used to confirm that the cat was also infected with a parasitic infection known as Haemabartonella felis. .

Coriolus versicolor supplementation*

i) On September 24th , 2004 for the fibrosarcoma, a decision was made to assist the immune system with *Coriolus versicolor* supplementation.

Days 1-15 -2 tablets per day (crush tablet (500 mg) and mix with meals)

Days 15-90 - 1 tablet per day (crush tablet (500 mg) and mix with meals)

ii) Concurrently, in the month of October, 2004, to contain the Haemabartonella felis infection, a course of tetracyclyine injections were given.

Observation 1 – November 9th, 2002 a further test determined that the parasitic infection was negative and the fibrosarcoma has reduced in size. *Coriolus versicolor* supplementation continued at 1 tablet per day as maintenance therapy.

Observation 2 -January 11th, 2003 - the parasitic infection is still negative and the cat has normal energy levels.

Observation 3 - April 5th 2003 - while maintaining supplementation at 1 tablet per day the cat continues to have normal energy levels.

Observation 4 - May 5th, 2003 – the cat continues on Coriolus supplementation of 1 tablet per day and the fibrosarcoma has faded.

Observation 5 - July 24th 2003 – the cat continues on Coriolus supplementation of 1 tablet per day and the fibrosarcoma has faded.

Comments:

1. For fibrosarcomas, *Coriolus versicolor* supplementation offers an adjuvant nutrition method (and cost effective tool). On the assumption that sarcomas may be linked to the presence of a virus, *Coriolus versicolor* supplementation assists the immune system and in turn allows the immune system to reduce the viral load and indirectly reduce the size of the sarcoma.

2. *Coriolus versicolor* may play a role in the support of the immune system against Haemabartonella felis infection. Usually, once an animal is susceptible to such an infection, recurrence is frequent. However, in this case, there was no recurrence of Haemabartonella felis infection while the cat was on Coriolus versicolor supplementation.

Conclusions:

In my experience with *Coriolus versicolor* supplementation I see the following three (3) immunonutrition applications in small pets:

1. Palliative Cancer Care - When an animal has been diagnosed with a palliative condition due to a tumor, as an alternative to "putting down" the animal I would recommend the following supplementation levels of *Coriolus versicolor*:

2. Feline Leukemia (FEVL) – In cases where FEVL virus has been diagnosed I would recommend a *Coriolus versicolor* supplementation level of 1.0 grams for 14 days, followed by 0.5 grams per day for as long as the FEVL virus remains detectable.

3. Age Related Cancer - After the age of seven years (7) in dogs and the age of ten (10) years in cats the respective immune systems require assistance. For example, cancer is age related in dogs, with golden retrievers, german shepherds, boxers and cocker spaniels being particularly vulnerable. Tumours are responsible for the deaths of more than 50% of animals older than 10 years of age. For this reason, with golden retrievers, german shepherds, boxes and cocker spaniels that are older than eight (8) years, I would recommend the following *Coriolus versicolor* supplementation (based on weight) as a "tonic" to assist the immune system.

Finally, it must be remembered that *Coriolus versicolor* supplementation is not a substitute for any existing medical procedure or medical product. *Coriolus versicolor* supplementation should be considered as complementary immunonutrition to support a small pet´s immune system.

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* Mycology Research Laboratories Ltd. supplied Corpet (*Coriolus versicolor*) for these case studies. For more information on Corpet please contact the website http://www.mycologyresearch.com . Corpet is a biomass of *Coriolus versicolor* which is composed of the mycelium and the primordia. Corpet is not PSK or Krestin or an extract of *Coriolus versicolor.*

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