Original Research

Myo- and cardiotoxic effects of the wild winter mushroom (*Flammulina velutipes*) on mice

Anne-Mari Mustonen[1,2], Maija Määttänen[3], Vesa Kärjä[4], Katri Puukka[5,6], Jari Aho[7], Seppo Saarela[8] and Petteri Nieminen[1,2]

[1]Institute of Biomedicine/Anatomy, School of Medicine, Faculty of Health Sciences, University of Eastern Finland, Kuopio FI-70211, Finland; [2]Department of Environmental and Biological Sciences, Faculty of Science and Forestry, University of Eastern Finland, Joensuu FI-80101, Finland; [3]Department of Food and Environmental Sciences, Faculty of Agriculture and Forestry, University of Helsinki, Helsinki FI-00014, Finland; [4]Department of Pathology, Kuopio University Hospital, Kuopio FI-70211, Finland; [5]NordLab Oulu, Oulu University Hospital, Oulu FI-90029, Finland; [6]Department of Clinical Chemistry, Faculty of Medicine, University of Oulu, Oulu FI-90014, Finland; [7]Municipal Veterinary Clinic of Joensuu, Joensuu FI-80110, Finland; [8]Department of Ecology and Genetics, Faculty of Science, University of Oulu, Oulu FI-90014, Finland

Corresponding author: Petteri Nieminen. Email: petteri.nieminen@uef.fi

Impact statement

This work is important to the field of functional foods, as it provides novel information about the potential myo- and cardiotoxic properties of an edible mushroom, *Flammulina velutipes*. The results are useful and of importance because *F. velutipes* is an actively cultivated mushroom and marketed as a health-promoting food item. The findings contribute to the understanding of the complexity of the balance between the beneficial and potentially harmful effects of mushroom consumption.

Abstract

Rhabdomyolysis (destruction of striated muscle) is a novel form of mushroom poisoning in Europe and Asia indicated by increased circulating creatine kinase levels. Particular wild fungi have also been reported to induce elevated creatine kinase activities in mice. *Flammulina velutipes* (enokitake or winter mushroom) is one of the most actively cultivated mushroom species globally. As it is marketed as a medicinal mushroom and functional food, it is important to examine whether it could induce potentially harmful health effects similar to some previously studied edible fungi. The present study examined the effects of *F. velutipes* consumption on the plasma clinical chemistry, hematology, and organ histology of laboratory mice. Wild *F. velutipes* were dried, pulverized, mixed with a regular laboratory rodent diet, and fed to the animals at 0, 3, 6, or 9 g/kg body mass/day for five days ($n = 6$/group). *F. velutipes* consumption caused increased activities of plasma creatine kinase and the MB-fraction of creatine kinase at 6–9 g/kg/d, indicating potentially deleterious effects on both skeletal and cardiac muscle. The plasma total and high-density lipoprotein cholesterol concentrations (at 9 g/kg/d) and white blood cell and lymphocyte counts (at 6–9 g/kg/d) decreased. Although the cholesterol-lowering properties of *F. velutipes* can be beneficial, the previously unexamined, potentially hazardous side effects of mushroom consumption (myo- and cardiotoxicity) should be thoroughly investigated before recommending this mushroom species as a health-promoting food item.

Keywords: Cardiotoxicity, creatine kinase, *Flammulina velutipes*, MB-fraction of creatine kinase, myotoxicity, rhabdomyolysis

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Introduction

Flammulina velutipes (Curtis ex Fries) Singer, also known as the winter mushroom, enokitake, velvet shank, and golden needle mushroom, is one of the most actively cultivated mushroom species globally.[1] In nature, it usually grows on trunks and stumps of broadleaf trees.[2] The fruiting of *F. velutipes* is triggered by low temperatures, and its natural distribution area is limited to Europe, northern Asia, and North America. It is recognized for its fine taste and good nutritional value.[3] *F. velutipes* has low calorie and fat contents but is rich in fiber, carbohydrates, proteins, and the essential fatty acids 18:2n-6 and 18:3n-3.[4,5] It also contains several bioactive compounds with potentially health-promoting effects and,

thus, it is marketed as a medicinal mushroom and functional food.[3]

Polysaccharides derived from *F. velutipes* have been reported to induce anti-cancer, anti-oxidant, and immunomodulatory effects as well as memory and learning improvement.[3] *F. velutipes* also contains components with potential influence against hypercholesterolemia, atherosclerosis, thrombosis, hypertension as well as anti-aging, anti-allergic, anti-microbial, and hepatoprotective properties.[3] At present, peer-reviewed information on the safety and efficacy of the use of *F. velutipes* extracts in human subjects is largely lacking, and considerable effort will be required to identify the molecules responsible for these alleged effects as well as their mechanism of action. We were unable to find clinical trials investigating the therapeutic potential of *F. velutipes*, and animal experiments would also be required to verify whether the effects seen *in vitro* can be reproduced *in vivo*.

The consumption of edible fungi has been considered safe based on tradition when a species has not caused any significant harmful effects. However, some species initially classified as edible have been later re-classified as toxic.[6] Fatalities due to a novel type of mushroom intoxication, rhabdomyolysis, have occurred in Europe and Asia after ingestion of wild mushrooms.[6–8] It is a syndrome where striated muscle is injured, leading to the release of intracellular muscle constituents into the circulation and extracellular fluid.[9] Rhabdomyolysis is delayed after *Tricholoma flavovirens/equestre* consumption and requires several consecutive meals, whereas the symptoms can appear in a few hours after a single meal of *Russula subnigricans*.[8] *T. flavovirens/equestre* was previously considered a valuable species, while *R. subnigricans* has apparently been consumed due to errors in species recognition. Indications of myo-, hepato-, and/or cardiotoxicity were also detected in mice after consumption of several wild and cultivated mushroom species for several consecutive days.[10–13]

F. velutipes has a long tradition of human consumption and cultivation[1] and while literature is scarce regarding its possible deleterious health effects, a case of anaphylaxis has previously been described.[14] In addition, an isolated and obscure case of *F. velutipes* poisoning with gastrointestinal symptoms has been reported.[15] As *F. velutipes* is marketed as a functional food, it is not only important to examine its health-promoting effects but also the potential adverse health consequences. The species is known to contain flammutoxin—a cardiotoxic and cytolytic protein causing, for instance, lysis of mammalian erythrocytes, electrocardiographic changes, decrease in blood pressure, and local irritation.[16,17] It is not considered toxic by oral administration.[18]

The aim of the present study was to investigate the effects of *F. velutipes* consumption on the plasma clinical chemistry, hematology, and tissue histology of mice to further assess its suitability as a functional food by screening not only for potential health benefits but also for side effects. As many edible mushroom species have been documented to induce myo-, cardio-, and/or hepatotoxic effects when consumed during several consecutive days,[10–13] it was hypothesized that *F. velutipes* could also have these adverse side effects on mice.

Materials and methods

F. velutipes were harvested in Sipoo, Southern Finland (60.282321 N, 25.163290 E) and Liperi, Eastern Finland (62.565643 N, 29.110207 E; 62.589732 N, 29.252305 E). These sites were distant from industrial establishments and heavy road traffic.[19] The specimens were identified by an expert based on literature.[20] The mushrooms were weighed and dried at +50°C for 8 h, pulverized, and mixed into a moisturized rodent diet (R36; Lactamin, Stockholm, Sweden). After careful mixing, the feeds were pelleted and dried at +50°C. The feed of the control group was processed similarly without adding the mushroom powder.

All procedures were approved by the Animal Care and Use Committee of the University of Joensuu, currently a part of the University of Eastern Finland. The experimental animals included 24 male NIH/S mice (age 140 ± 10 d, body mass [BM] 36.3 ± 0.72 g) from the laboratory colony of the University that were housed singly in standard wire cages ($42 \times 22 \times 15$ cm) with wood shavings for bedding at 21 ± 1°C and 12L:12D. The mice were divided into four study groups as follows: control group ($n = 6$), group receiving dried *F. velutipes* at 3 g/kg BM/d ($n = 6$), group receiving dried *F. velutipes* at 6 g/kg BM/d ($n = 6$), and group receiving dried *F. velutipes* at 9 g/kg BM/d ($n = 6$) for five days. The selection of the doses and duration of the exposure was based on previous studies examining the myotoxic effects of different fungi[10,11,13] to enable direct comparison of the present results to previous ones. The animals had free access to food and water.

The mice were weighed at the beginning of the feeding trial and at sampling. Their food and water consumptions were followed. At the end of the study, the mice were fasted for 30 min and euthanized by an overdose of diethyl ether. The blood samples were obtained by cardiac puncture with sterile needles and syringes into test tubes containing ethylenediaminetetraacetic acid. After the determination of the blood count, the plasma was separated by centrifugation at 4000*g*, frozen with liquid nitrogen, and stored at –70°C. The liver, spleen, kidneys, adrenals, testes, heart, and the left *quadriceps femoris* muscle were dissected, weighed, and frozen or stored in neutral formalin fixative for histological analyses.

The blood count was determined with the Vet abc Animal Blood Counter calibrated to the murine hematologic profile (ABX Hematologie, Montpellier, France) at the Municipal Veterinary Clinic of Joensuu. Most of the variables of plasma clinical chemistry were determined with the Technicon RA-XT analyzer (Swords, Ireland) using the reagents of the Randox Laboratories Ltd (Crumlin, UK) as outlined previously.[21–23] The activity of aspartate aminotransferase was measured by a kinetic IFCC modified method (ADVIA 1800, Siemens Healthcare Diagnostics, Tarrytown, NY, USA) at the Oulu University Hospital.

The adrenal catecholamines were determined with high-performance liquid chromatography based on a previously described method.[24] The histological samples were

dehydrated, embedded in paraffin, and cut into sections that were attached to glass slides and stained with hematoxylin–eosin. The slides were examined by a consultant pathologist unaware of group assignment by using conventional light microscopy (Leica DM LB, Leica Microsystems, Heerbrugg, Switzerland).

Comparisons between the study groups were performed with the generalized linear model (IBM SPSS *v*21.0 software, IBM, Armonk, NY, USA) under the supervision of our resident statistician. The model was performed with normal probability distribution, and the examined parameter was selected as the dependent variable and the dose group as the model factor. Histological data were tested with the Fisher's exact test. The P value <0.05 was considered statistically significant. The results are presented as the mean ± SE.

Results

The actual amounts of *F. velutipes* ingested were close to the desired values in all groups (Table 1). There were no visual signs of toxicity (diarrhea, myoglobinuria, lethargy, etc.) in any of the experimental animals. The relative food and water intakes were similar between the study groups, but the energy balance was slightly negative for the control and 9 g/kg/d groups and positive for the 3 and 6 g/kg/d groups.

The activities of plasma creatine kinase (CK) and the MB-fraction of CK (CK-MB) increased in the 6 and 9 g/kg/d groups and the same was observed for plasma bilirubin concentrations at 3 and 6 g/kg/d (Table 2). Decreases were documented for plasma total cholesterol (at 9 g/kg/d), high-density lipoprotein (HDL) cholesterol (at 9 g/kg/d), and low-density lipoprotein (LDL)

Table 1. The amount of winter mushroom consumption and its effects on the general variables of the mice (mean ± SE).

	Control	3 g/kg/d	6 g/kg/d	9 g/kg/d	*P*
Amount ingested, g/kg/d[a]	—	2.8 ± 0.2[b]	5.9 ± 0.2[b]	8.5 ± 0.4[b]	<0.0004
Fresh mushroom, g/kg/d[a,c]	—	18.7 ± 1.0[b]	39.5 ± 1.4[b]	56.6 ± 2.7[b]	<0.0004
Calculated human dose, kg/d[a,d]	—	1.1 ± 0.1[b]	2.4 ± 0.1[b]	3.4 ± 0.2[b]	<0.0004
Corrected human dose, g/d[a,e]	—	91 ± 5[b]	193 ± 7[b]	276 ± 13[b]	<0.0004
BM change, %	−1.5 ± 0.9	0.7 ± 0.6[b]	1.5 ± 0.4[b]	−1.2 ± 0.7	0.001
Water intake, ml/g BM	1.3 ± <0.1	1.3 ± 0.1	1.2 ± 0.1	1.3 ± 0.1	0.900
Food intake, g/g BM	0.7 ± <0.1	0.7 ± <0.1	0.7 ± <0.1	0.7 ± <0.1	0.569
Liver mass/BM, %	5.14 ± 0.16	6.35 ± 0.83[b]	5.03 ± 0.99	5.08 ± 0.09	0.047
Kidneys mass/BM, %	1.86 ± 0.07	2.01 ± 0.16	1.88 ± 0.04	1.88 ± 0.06	0.576
Spleen mass/BM, %	0.44 ± 0.03	0.44 ± 0.06	0.38 ± 0.04	0.41 ± 0.03	0.769
Adrenals mass/BM, ‰	0.16 ± 0.02	0.16 ± 0.02	0.17 ± 0.03	0.14 ± 0.01	0.759
Testes mass/BM, %	0.47 ± 0.02	0.42 ± 0.05	0.48 ± 0.03	0.47 ± 0.03	0.429

BM: body mass.
[a]All dose groups differ significantly from each other (generalized linear model, $P < 0.05$).
[b]Differs significantly from the controls (generalized linear model, $P < 0.05$).
[c]The calculated dose of fresh mushroom.
[d]The calculated dose of fresh mushroom for a 60-kg person.
[e]The calculated dose of fresh mushroom for a 60-kg person normalized to body surface area.[25]

Table 2. The effects of winter mushroom consumption on the plasma clinical chemistry of the mice (mean ± SE).

	Control	3 g/kg/d	6 g/kg/d	9 g/kg/d	*P*
Glucose, mmol/l	13.8 ± 1.4	14.1 ± 1.1	12.7 ± 0.9	13.4 ± 0.8	0.702
TAG, mmol/l	2.05 ± 0.03	2.49 ± 0.33	2.48 ± 0.15	1.94 ± 0.21	0.126
Total cholesterol, mmol/l	2.5 ± 0.1	2.4 ± 0.1	2.5 ± 0.1	2.2 ± 0.1[a]	0.040
HDL-cholesterol, mmol/l	1.90 ± 0.06	2.00 ± 0.13	1.99 ± 0.07	1.58 ± 0.08[a]	<0.0004
LDL-cholesterol, mmol/l	0.18 ± 0.04	0.18 ± 0.01	0.13 ± 0.01[a]	0.14 ± 0.01	0.010
Creatinine, μmol/l	50 ± 1	45 ± 2	47 ± 2	49 ± 12	0.941
Total protein, g/l	47 ± 3	48 ± 2	47 ± 1	44 ± 1	0.283
Urea, mmol/l	7.6 ± 0.5	8.8 ± 0.6	8.8 ± 0.4	8.3 ± 0.5	0.379
Uric acid, μmol/l	253 ± 39	283 ± 36	223 ± 18	230 ± 16	0.262
Ammonia, μmol/l	840 ± 7	857 ± 6	854 ± 6	859 ± 14	0.472
CK, U/l	133 ± 34	192 ± 38	273 ± 60[a]	224 ± 22[a]	0.015
CK-MB, U/l	433 ± 105	606 ± 94	817 ± 160[a]	688 ± 62[a]	0.012
ALT, U/l	52 ± 4	83 ± 16	68 ± 11	61 ± 10	0.289
AST, U/l	96 ± 3	157 ± 23	146 ± 24	128 ± 14	0.170
Bilirubin, μmol/l	5.2 ± 0.6	7.8 ± 1.4[a]	8.6 ± 1.1[a]	6.1 ± 0.7	0.036
TAS, mmol/l	1.9 ± 0.08	2.1 ± 0.09	2.1 ± 0.04	2.0 ± 0.04	0.363

TAG: triacylglycerols; HDL: high-density lipoprotein; LDL: low-density lipoprotein; CK: creatine kinase; CK-MB: MB-fraction of creatine kinase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; TAS: total antioxidant status.
[a]Differs from the controls (generalized linear model, $P < 0.05$).

Table 3. The effects of winter mushroom consumption on the blood count of the mice (mean ± SE).

	Control	3 g/kg/d	6 g/kg/d	9 g/kg/d	*P*
WBC, $10^3/mm^3$	4.9 ± 0.4	5.1 ± 0.5	3.7 ± 0.5[a]	3.9 ± 0.3[a]	0.010
RBC, $10^6/mm^3$	8.7 ± 0.2	8.8 ± 0.5	9.0 ± 0.1	9.0 ± 0.1	0.653
HGB, g/l	139.2 ± 2.4	142.3 ± 7.7	145.8 ± 1.4	140.8 ± 2.0	0.648
HCT, %	45.9 ± 1.0	46.6 ± 2.8	48.3 ± 0.5	46.7 ± 0.8	0.686
MCV, fl	53.0 ± 0.5	53.0 ± 0.4	53.3 ± 0.3	52.6 ± 0.2	0.589
MCH, pg	16.1 ± 0.2	16.3 ± 0.2	16.1 ± 0.1	15.8 ± 0.1	0.066
MCHC, g/l	303.5 ± 1.5	306.2 ± 3.1	302.6 ± 0.9	301.6 ± 1.5	0.302
RDW, %	13.4 ± 0.1	14.2 ± 1.0	13.3 ± 0.2	13.0 ± 0.1	0.314
LYM, %	78.3 ± 2.7	70.3 ± 2.3[a]	78.0 ± 2.2	79.2 ± 1.7	0.008
MON, %	7.4 ± 0.9	9.6 ± 0.7[a]	6.4 ± 0.5	7.0 ± 0.5	0.001
GRA, %	14.4 ± 1.9	20.1 ± 1.7[a]	15.6 ± 1.8	13.9 ± 1.3	0.015
LYM, $10^3/mm^3$	3.8 ± 0.2	3.5 ± 0.3	2.8 ± 0.3[a]	3.0 ± 0.3[a]	0.007
MON, $10^3/mm^3$	0.33 ± 0.06	0.45 ± 0.07	0.18 ± 0.05[a]	0.22 ± 0.04	0.001
GRA, $10^3/mm^3$	0.82 ± 0.13	1.17 ± 0.16[a]	0.77 ± 0.16	0.65 ± 0.06	0.019

WBC: white blood cell count; RBC: red blood cell count; HGB: hemoglobin; HCT: hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW: red cell distribution width; LYM: lymphocytes; MON: monocytes; GRA: granulocytes.
[a]Differs from the controls (generalized linear model, $P < 0.05$).

Table 4. The effects of winter mushroom consumption on the tissue biochemistry of the mice (mean ± SE).

	Control	3 g/kg/d	6 g/kg/d	9 g/kg/d	*P*
Liver fat, %	4.9 ± 0.27	6.6 ± 0.34[a]	4.3 ± 0.17	5.6 ± 0.29	<0.0004
Liver triacylglycerols, mg/g	6.5 ± 0.94	7.2 ± 1.02	8.0 ± 1.26	6.8 ± 0.89	0.711
Liver glycogen, μg/mg	3.4 ± 0.27	11.3 ± 2.69[a]	4.9 ± 0.26	5.2 ± 0.68	<0.0004
Muscle glycogen, μg/mg	1.1 ± 0.29	1.6 ± 0.18	2.2 ± 0.58	2.4 ± 0.53	0.060
Liver protein, μg/mg	225 ± 4	210 ± 9	211 ± 5	215 ± 5	0.197
Muscle protein, μg/mg	205 ± 5	215 ± 9	210 ± 6	222 ± 8	0.298
Adrenal adrenaline, ng/mg	2699 ± 246	3146 ± 247	2446 ± 297	2840 ± 268	0.252
Adrenal noradrenaline, ng/mg	1342 ± 114	1530 ± 169	1341 ± 114	1384 ± 149	0.697
Adrenal dopamine, ng/mg	37 ± 5	41 ± 3	34 ± 3	36 ± 5	0.632

[a]Differs from the controls (generalized linear model, $P < 0.05$).

cholesterol levels (at 6 g/kg/d). The other variables of clinical chemistry, such as creatinine and transaminases, remained unresponsive to *F. velutipes.*

Reductions in the white blood cell counts were observed in the 6 and 9 g/kg/d groups with similar changes for the lymphocyte (at 6–9 g/kg/d) and monocyte counts (at 6 g/kg/d; Table 3). The 3 g/kg/d group showed elevated absolute and relative numbers of granulocytes and relative number of monocytes, reduced relative number of lymphocytes, and higher relative liver mass together with elevated liver lipid and glycogen concentrations (Tables 1, 3 and 4). *F. velutipes* did not influence the masses of the other organs or the other parameters of the blood count and tissue biochemistry. In the histological samples, there were no indications of *F. velutipes*-induced necrosis or inflammation in the skeletal muscle, heart, liver, or kidneys (data not shown).

Discussion

Several cultivated and traditionally consumed wild mushrooms can induce myo-, hepato-, and/or cardiotoxic effects on mice.[7,10–13] The increased CK activity is the most common finding accompanied with elevated CK-MB, transaminase, bilirubin, and/or creatinine levels depending on the species and dosage. Data from animal experiments are supported by several human cases requiring hospitalization due to rhabdomyolysis with elevated CK and transaminase activities. These occurred in Europe and Asia after consumption of wild mushrooms.[6–8,26–28] The actual chemical substances causing muscle toxicity remain unknown for the edible fungi and *T. flavovirens/equestre* but *R. subnigricans* is known to contain cycloprop-2-ene carboxylic acid that triggers rhabdomyolysis.[29]

The main finding of the present study was that *F. velutipes* consumption induced elevated levels of plasma CK at 6–9 g/kg/d. This result is similar to those obtained from several other wild fungi, including highly appreciated species,[10–12] and the cultivated shiitake *Lentinula edodes*.[13] Myotoxic mushrooms can also induce cardiac muscle injury leading to cardiopulmonary complications,[6] and the elevated activity of plasma CK-MB at 6–9 g/kg/d could be an indicator of cardiotoxicity. *F. velutipes* is known to contain a cardiotoxic protein (flammutoxin) but, so far, it has not been considered effective by oral administration.[16–18] It must be acknowledged that the increases in the CK and CK-MB activities we observed were quite modest and no visible damage was detected in the light microscopy. The elevated activities do not necessarily signal cell death but could have derived from

increased permeability of cell membranes[30] and present a very early and asymptomatic stage of intoxication.

Consumption of wild fungi has previously increased transaminase activities in mice[11] and humans[26,28] suggesting potential liver damage. Transaminase activities were not elevated in the present study, but we noticed increased plasma bilirubin concentrations at 3–6 g/kg/d that could not be reproduced at 9 g/kg/d,[12,13] which may be related to interindividual differences in the sensitivity. It must be recalled that the studied mushroom species contains a complex combination of bioactive compounds and, for instance, a water-soluble polysaccharide, FVP2, has been isolated from *F. velutipes* mycelium and proposed to induce hepatoprotective activity against CCl_4 intoxication.[31] Increased bilirubin may also derive from the breakdown of myoglobin leaked from the injured muscle.[9] The exposure of the kidney to myoglobin could lead to acute renal failure that is a potential complication of rhabdomyolysis, but the plasma creatinine, urea, or uric acid levels of the studied mice were not elevated due to *F. velutipes* consumption.[10,12]

The white blood cell and lymphocyte counts reduced after *F. velutipes* consumption at 6–9 g/kg/d. This finding differs from previous literature, according to which *F. velutipes* extracts activate the immune system by increasing the proliferation of lymphocytes and the secretion of cytokines.[32,33] Components responsible for these effects include a fungal immunomodulatory protein FIP-fve and polysaccharides, such as FVP I-A. Neither did the present study find any effects of *F. velutipes* consumption on the total antioxidant status of the mice plasma. This also contradicts previous literature, according to which this mushroom contains several bioactive components, such as polysaccharides, phenolic compounds, and rhamnose sugar, with antioxidant activity.[3,33,34] With no clear standardization of research procedures, it is expected that the observations will remain somewhat diverse also in the future. Differences in the responses may result from the use of cultivated *vs.* wild mushrooms and different developmental stages.[3,35] In addition, the processing and administration routes of fruiting bodies and/or extracted compounds *in vivo* or performing the experiments *in vitro* could lead to different outcomes.

Many mushroom species have been reported to induce hypocholesterolemic effects on laboratory rodents and *F. velutipes* is no exception.[34,36,37] As expected, the present study reproduced these findings with decreased plasma total and HDL-cholesterol concentrations (12–17%) at 9 g/kg/d of *F. velutipes* consumption. The influence on LDL-cholesterol reached significance at 6 g/kg/d. As proposed by Fukushima *et al.*,[36] these effects could be transmitted through enhanced cholesterol excretion in feces and via elevated LDL-receptor mRNA levels in the liver. In addition to fiber,[36] lovastatin present in *F. velutipes* is one potential agent responsible for the cholesterol-lowering effects.[38] While the hypocholesterolemic effect is clear and potentially beneficial in hyperlipidemic patients, the possibility of side effects needs to be assessed in more detail before the use of this species in health-promotion can be recommended without any caution.

As stated above, *R. subnigricans* contains cycloprop-2-ene carboxylic acid that triggers rhabdomyolysis,[29] but the myotoxic substances in the edible fungi remain unknown. *F. velutipes* contains a cardiotoxic protein, flammutoxin, but it is not considered effective by oral administration, and its toxicity can be completely eliminated by heating.[16–18] If the myotoxic substance in *F. velutipes* was a protein or a shorter peptide, it could be assumed to be at least partly inactivated or destroyed by cooking, although it must be mentioned that the lethal mushroom-derived oligopeptides, amatoxins, are thermostable.[39] It should also be acknowledged that the present experiment did not include a study group consuming well-cooked mushrooms, although the fungi were heated during the preparation of the experimental feeds. However, as *F. velutipes* is consumed not only cooked but also raw,[40] the results remain relevant and could actually underestimate the potential effects of, e.g. fresh salads containing *F. velutipes*.

To conclude, the results of the present experiment support existing literature on the myotoxic effects of wild mushrooms when consumed for several consecutive days.[10–12] These toxic effects are presumably not species-specific as they have been reported for many species. However, the myotoxicity of *F. velutipes* seems to be modest and to require individual sensitivity and repeated meals to manifest itself. The equivalent human dose would be 190–280 g of fresh *F. velutipes* for five days when normalized to body surface area, which is theoretically feasible but probably not a common way to consume this mushroom species. The balance between the beneficial and potentially harmful effects of mushroom consumption is complex and it is difficult to classify a mushroom species only as useful or harmful. Based on the present results, some caution is warranted regarding the marketing of *F. velutipes* as a medicinal mushroom and functional food.

Authors' contributions: PN and A-MM designed and coordinated the study. PN, A-MM, and MM performed the experiment and collected the samples. PN, A-MM, MM, KP, SS, and JA performed the laboratory analyses and VK carried out the histological analyses. PN performed the statistical analyses. A-MM drafted the manuscript. All authors revised the draft critically and read and approved the final submitted manuscript.

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