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Lipid profile and glycemic response of rats fed on a semi-purified diet supplemented with *Agaricus brasiliensis* mushroom

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ABSTRACT. The search for more healthy alimentary habits has stimulated the study of new food sources. Edible mushrooms, such as the genus *Agaricus*, may be underscored. Current assay evaluates the dietary influence of diets supplemented with the mushroom *Agaricus brasiliensis* on the metabolic profile of lipids and glycemic behavior in rats. A trial with 28 male Wistar rats in 4 groups with 7 rats each was carried out during 32 days. Diets given to these groups were AIN-93 (CAS) for Group 1; AIN-93 with 1% cholesterol (CAS + COL) for Group 2; the same for Group 3 and 4, but supplemented with or without mushrooms [(COG) and (COG + COL) respectively]. Analyses of all samples for cholesterol, triacylglycerols, hepatic cholesterol and hepatic lipids on the 32nd day showed that *Agaricus* mushroom modified the lipid profile, reduced total cholesterol by 16% and triacylglycerols by 26.9% and increased HDL by 60.2%, coupled to reduction of lipid and cholesterol levels in the liver and a higher elimination of lipids in the stool. Glycemic curve decreased significantly between fifteen and sixty minutes in rats fed on *Agaricus* supplemented diets.

Keywords: himematsutake. lipid profile. cholesterol. *Agaricus brasiliensis.*

Perfil lipídico e resposta glicêmica de ratos alimentados com uma ração semi-purificada suplementada com o cogumelo *Agaricus brasiliensis*

RESUMO. A busca por hábitos alimentares saudáveis tem incentivado o estudo de novas fontes alimentares. Destacam-se os cogumelos comestíveis, como os do gênero *Agaricus.* Este trabalho avaliou a influência da ração semi-purificada, suplementada com o cogumelo *Agaricus brasiliensis* no perfil de lipídios, em ratos. Foi realizado experimento com 28 ratos machos Wistar, em 32 dias. Os animais foram separados em quatro grupos de sete dos quais o primeiro recebeu dieta AIN-93 (CAS), o segundo recebeu dieta AIN-93 adicionada de 1% de colesterol (CAS + COL) e o terceiro e o quarto grupos foram alimentados com dieta AIN-93 suplementada com cogumelos sem (COG) e com (COG + COL) adição de colesterol a 1%, respectivamente. No 32.º dia, amostras foram coletadas para análises de colesterol, triglicerídeos, colesterol hepático da gordura hepática. O estudo mostrou que *Agaricus brasiliensis* influenciou o perfil lipídico, diminuindo o colesterol total (-16%) e os triglicérides (- 26,9%), além de aumentar o HDL (+ 60,2%). É possível afirmar que nutrientes contidos em *Agaricus brasiliensis* são moduladores do perfil lipídico de ratos, diminuindo a deposição de lipídios hepáticos e aumentando a sua eliminação fecal. A curva glicêmica mostrou declínio significativo entre quinze e sessenta minutos em ratos alimentados com dieta contendo o cogumelo.

Palavras-chave: himematsutake. perfil lipídico. colesterol. *Agaricus brasiliensis*.

Introduction

The amount of dietary fiber in Brazilian diets has decreased significantly due to a reduction in the consumption of traditional fresh food, mostly those of vegetable origin. Changes in the eating habits by different social classes in Brazil have been associated with rising incomes and changing life patterns and behavior (Instituto Brasileiro de Geografia e Estatística [IBGE], 2009). In the wake of economic development during recent years, there is a rapid advance of morbidity and mortality caused by diabetes, obesity and cardiovascular diseases associated with consumption of processed foods, rich in simple carbohydrates but high in fat and calories with low dietary fiber. One of the great challenges of the century in developed and developing countries has been avoiding the above-mentioned transition in the population´s dietary patterns. In the case of diet, several studies have shown that the components of dietary fiber, particularly soluble ones, may influence lipid metabolism in direct association between fiber intake and reduced risk of cardiovascular diseases (Estruch et al., 2009).

There are several places in humans or animals where the dietary fiber may interact with physiological structures, such as the intestinal lumen, by modifying the kinetics of nutrient absorption through changes in solubility and pH or through post-absorptive ones such as modulation of pancreatic secretion, lipoprotein metabolism, secretion of bile acids or the effects of colonic fermentation by-products in the hepatic synthesis of cholesterol (Wong, De Souza, & Kendalli, 2006).

Studies on animals and humans have shown that non-digestible polysaccharides, such as beta glucans, decrease plasma cholesterol, particularly in hypercholesterolemic subjects, and attenuate glycemic response and post-meals insulin, which allows their use in prevention and control of chronic non-transmitted diseases (Smiderle et al., 2010). Some fungi of the Basidiomycete family lower the concentration of blood glucose and serum cholesterol. It has been reported that Hiratake (*Pleurotus ostreatus*) reduces serum cholesterol in rats, and that the fruit of straw mushroom (*Volvariella volvacea*) reduces cholesterol and stabilizes blood glucose in hypercholesterolemic hamsters, suggesting the influence of polysaccharides present in their composition on the hepatic synthesis and fecal excretion of cholesterol (Henry, Eswaran, & Vigilanbiah, 2007). Edible mushrooms contain high proportions of carbohydrates, including chitin, glycogen, trehalose and mannitol. They also contain fiber, β-glucans, hemicelluloses and pectic substances. Additionally, glucose, mannitol and trehalose constitute abundant sugars in cultivated edible mushrooms, but fructose and sucrose are found in low amounts. Moreover, mushrooms have great nutritional rates since they are quite rich in protein, with an important content of essential amino acids, B complex vitamins and minerals, coupled to poor fat concentration but with an excellent profile of fatty acids (Valverde, Hernández-Perez, & Paredes-López, 2015).

Food matrix, such as *Agaricus brasiliensis* mushroom (*A. brasiliensis*), a native species of the Atlantic Rain Forest, has been commercially exploited, although little is known on the true range of its properties (Henriques, Simeone, & Amazonas, 2008). According to Kalac (2013), mushroom polysaccharides have homo- and hetero-glucans of the glycoside β (1-6), with functional properties, acting on the gastrointestinal tract and the immune system. β-glucans are the main polysaccharides in mushrooms and around half of the fungal cell wall mass is constituted by β-glucans. They favor anticancer, immune-modulating, anticholesterolemic, antioxidant, and neuro-protective activities of many edible mushrooms (Reis, Barros, & Martins, 2012; Lima, Cordova, Nobrega, Funghetto, & Kamikowski, 2011).

From the nutritional point of view, the full polysaccharide contents of *Agaricus brasiliensis* suggest further in-depth investigation on the influence of their diet consumption on glucose and lipid metabolic profile (Loreto, González-Franco, Soto-Parra, & Montes-Domínguez, 2008). Although dietetic fiber is acknowledged as important in the modulation of lipid metabolism (Niwa, Tajiri, & Higashino, 2011), the specific mechanisms by which they reduce the concentration of plasma cholesterol and modulate the percentage of fat stored in the liver are still controversial (Sang et al., 2010).

Since *A. brasiliensis* is a food matrix with potential consumption of dietary fiber in mixed diets, current study evaluated the influence of dietary consumption of a diet based on *A. brasiliensis* mushrooms on lipid metabolic profile and its effect on liver tissue architecture and on blood glucose in a model formed by Wistar rats.

Material and methods

Experimental diets

Four experimental diets were formulated, namely, CAS (Casein Standard Diet), COG (Casein Standard Diet plus *A. brasiliensis*), CAS + COL (Casein Standard Diet with 1% cholesterol) and COG + COL (Casein Standard Diet plus *A. brasiliensis* with 1% cholesterol). The CAS was prepared according to ingredients and amounts recommended by AIN-93 (Reeves, Nielsen, & Fahey, 1993). COG diet was prepared from a matrix of the dried fruit body of *A. brasiliensis*, from Agaricus Pilar Group (OPE) supplied by the Brazilian Agricultural Research Corporation (Embrapa). It was ground and incorporated into the base formulation of the AIN-93 for nutrients other than the protein source. CAS + COL was based on AIN-93 with 1% cholesterol, whereas COG + COL was prepared with *A. brasiliensis* with 1% cholesterol.

Diets were iso-nitrogenous and iso-caloric, while recommendations by the National Agency of Sanitary Surveillance (Zanebon & Pascuet, 2008) were complied with as quality standard for diet preparation.

Determination of diet composition

Total protein was determined in a 0.5 g sample by micro-Kjeldahl method which quantified the nitrogen content. The protein concentration was calculated by multiplying the percentage of total nitrogen by the conversion factor 6.25. Moisture was determined by an approximately 5 g sample kept in an oven at 105°C for 12 hours. Ash rate, representing the total of minerals, was determined by calcination of approximately 2 g of the sample in triplicate in a muffle furnace at 550°C for three hours. Lipids (ether extract) were determined by

analyzing moisture and using ethyl ether as a solvent extractor for six hours. Ether extract obtained was placed in an oven at 70°C for one hour to remove residual solvent, cooled in a dry area and weighed. With the exception of the analysis of dietary fiber and carbohydrates, the nutritional composition was performed in triplicate, following the official method by Adolfo Lutz Institute (Zanebon & Pascuet, 2008). The insoluble and soluble fractions and total enzymatic method were done according to Prosky, Asp, Schweizer, Devries and Furda (1992) with about 1g of the sample, in quadruplicate. The method consists in hydrolyzing protein with protease, followed by hydrolysis of starch with thermal stable alpha-amylase and amyloglucosidase (glucoamylase) enzymes.

Biological assay

Twenty-eight weaned male Wistar rats (n = 28), weighing 60 ± 2.53 g, were randomly divided into four groups of seven animals each and kept in semi-metabolic cages throughout the experiment. Each group of rats was given a specific diet, with water *ad libitum* during the 32 days of the experiment. Rats were given a washout period of 3 days after consuming the basal diet prior to the experiment. Control rats were fed only on basal diet (CAS), supplemented for the other three groups by the above-mentioned diets [Agaricus (COG group), at 1% cholesterol (CAS + COL group) and at 1% cholesterol over Agaricus (group COG + COL)]. Food intake and weight of rats were monitored at the end of every 2 days.

Determination of plasma lipid profile

Blood samples to determine plasma lipids were first collected from the tail tip vein and by cardiac puncture at the final stage of the experiment. Plasma was separated by centrifugation at 3000 rpm for 15 minutes using a centrifuge table brand CELM® and tested with commercial kits LABTEST® with spectrophotometer Beckman® DU 700 series (Brea, CA). Total cholesterol and triglycerides (Bucolo & David, 1973) were determined by enzymatic methods. HDL cholesterol was determined after precipitation of chylomicrons; low density lipoprotein (LDL) and very low density lipoproteins (VLDL) were calculated by the phosphotungstic acid/magnesium chloride technique.

Determination of lipids from liver and stools

The livers, removed at the end of the experiment to determinate lipids, were dried at 50°C for 1-2 hours, ground and subjected to lipid extraction, using 2:1 chloroform-methanol solution in the extraction apparatus (Matos et al., 2005). Similarly, animal stools were collected throughout the experiment and dried in an oven at 60°C for 2 days, crushed and subjected to lipid extraction using a 2:1 chloroform-methanol solution in a Soxhlet apparatus. Residues were then dried in an oven at 105°C for 1 hour and weighed on an analytical balance. The extraction residue was then dried on a stove at 105°C for 1 hour and weighed on an analytical balance.

Histological analysis of liver tissue

Livers were stored in vials with 10% formalin and placed on histologic slides in paraffin-embedding technique stained with hematoxylin-eosin. Reading, done by computerized microscope Olympus BX-40® with Image Pro Plus 4.0 software for morphometry, was based on the count of empty spaces (fat cells) through the hepatic parenchyma with the consequent calculation of lesion area percentage.

Analysis of blood glucose

All animals in the experiment were evaluated five times at 0, 1st, 2nd, 3rd and 4th week follow-up. Prior to glucose evaluation, the animals fasted for 8 hours. A solution of D-glucose (2.5 g kg^{-1} body weight) was administered orally in bolus with a 50 mL squirt, with subsequent measurement of glucose and construction of the glucose curve data at times 0, 5, 15, 30, 60, 90 and 120 minutes after sample collection. Blood samples were collected from the tail vein of the rats and analyzed by glucose oxidase method to monitor glucose levels in the plasma. Further, 2 μL of blood was mixed with 200 μL of enzyme solution and absorbance was read at 492 nm wavelength in a UV-VIS Spectrophotometer (Beckman Coulter®, DU 700 Series, USA).

The area under glycemic curve was calculated by the sum of increments of the curve, as follow:

$$\text{Area under curve} = \left(A + B + C + \frac{D}{2}\right)\text{t} + \frac{(D+E)T}{2} + \frac{E^2T}{2\,(E+F)}$$

where:

A, B, C, D, E and F are the glycemic increments under the curve;

T and t represent time after D-glucose solution ingestion.

Statistical analysis

The statistical analysis/description of all data obtained in current study was performed with SPSS 13.0®. Total data variation from each treatment was decomposed by one-way ANOVA. Post-hoc comparisons were made by Tukey's test to detect differences between means at 95% significance level.

Results and discussion

Table 1 shows the analytical data of the experimental diets, with the characteristics of each treatment.

Table 1. Analysis of Chemical Composition of experimental diets*.

Experimental** Diets	Moisture (g 100^{-1} g)	Dry Ash (g 100^{-1} g)	Lipids (g 100^{-1} g)	Protein (g 100^{-1} g)	Dietary Fiber (g 100^{-1} g)	Nifext (g 100^{-1} g)	Calories (kcal 100^{-1} g)
CAS	6.58 (0.92)	3.64 (0.43)	6.8 (1.35)	9.9 (1.31)	3.37 (0.54)	69.71 (2.37)	379.64 (21.33)
CAS + COL	5.98 (0.91)	3.09 (0.27)	18.3 (2.01)	9.6 (1.57)	3.16 (0.39)	59.87 (2.72)	442.58 (33.22)
COG	5.55 (0.69)	2.78 (0.39)	8.2 (1.52)	9.2 (1.55)	8.41 (1.11)	65.86 (3.13)	374.04 (24.27)
COG + COL	6.12 (0.75)	3.21 (0.51)	17.6 (1.85)	9.4 (1.27)	8.03 (0.98)	55.64 (3.81)	418.56 (36.96)

*Mean rates and standard deviation of five replicates. **CAS = Casein Standard Diet; CAS + COL = Casein Standard Diet plus 1% Cholesterol; COG = Casein Standard Diet plus *Agaricus* and COG + COL = Casein Standard Diet plus *Agaricus* and 1% Cholesterol; Nifext = Nitrogen free extract.

Table 2 shows dietary intake and resulting weight gain by rats fed on different diets. The table reveals that the addition of 1% cholesterol to standard semi-purified diets, with or without the addition of mushroom *A. brasiliensis*, did not significantly affect the pattern of food intake nor influence rats´ growth and development.

Table 2. Dietary Intake and weight gain of rats fed on Casein Standard Diet (CAS); Casein Standard Diet plus 1% Cholesterol (CAS + COL); Casein Standard Diet plus *Agaricus* (COG) and Casein Standard Diet plus *Agaricus* and 1% Cholesterol (COG + COL)*.

Treatment	Dietary Intake 32 days (g)	Weight Gain 32 days (g)
CAS	426.81 (9.91) [a]	91.86 (10.48) [a]
CAS + COL	423.18 (10.75) [a]	88.34 (6.57) [a]
COG	419.63 (11.32) [a]	89.41 (5.63) [a]
COG + COL	421.45 (8.79) [a]	88.92 (7.12) [a]

*Different letters on each column demonstrate statistical significant differences at p <!0.05 level. Results in each column represent means and standard deviation.

Figure 1 shows comparative plots of glycemic profiles obtained from rats fed on different diets.

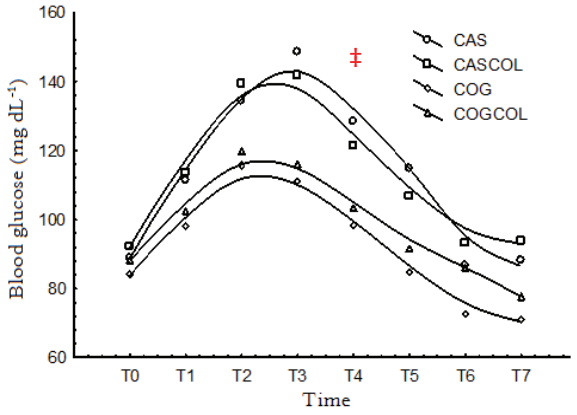

Figure 1. Comparison between glycemic profiles obtained from rats fed on Casein Standard Diet, (CAS); Casein Standard Diet plus 1% Cholesterol (CAS + COL); Casein Standard Diet plus *Agaricus* (COG) and Casein Standard Diet plus *Agaricus* and 1% Cholesterol (COG + COL). *† ‡ Different symbols signify statistically significant differences at p !<! 0.05 level.

There is a clear attenuation of blood glucose levels when diet contains *A. brasiliensis*. Data revealed that it decreased between fifteen and sixty minutes after rats were fed on *Agaricus*-added diets. This effect on the diet with n †cholesterol is significant when compared to that in rats fed on a casein standard diet. Rats fed on diet with mushrooms and cholesterol showed small changes in blood glucose when compared to those fed on casein standard diet only (Figure 1). The direct correlation between the sustained increase in blood glucose and levels of total cholesterol, plasma triglycerides and decreased HDL strongly suggests the influence of dietary fiber factor on the consumed diet. Oh et al. (2010) reported that streptozotocin had caused the same attenuation effect on diabetic rats. Effect did not merely consist of low final blood glucose concentration but also in low growth of the glycemic curve during the first 30 minutes of a trial in animals fed on fiber-rich diets from mushrooms.

Table 3 lists the levels of total cholesterol and triglycerides. It may be observed that these parameters are significantly affected when cholesterol is added to the standard casein diet with the recommended amounts of dietary fiber in the form of microcrystalline cellulose. However, when *A. brasiliensis* mushroom is added to the formulation, the levels tend to decrease significantly, comparable to those found in diets without the addition of cholesterol. Further, the comparison of results of the treatment groups CAS and CAS + COL shows that the model rats used in this experiment were responsive to changes in the diet (control group). In other words, there is a metabolic response proportional to the concentration of cholesterol in the diet, such as its presence with increased concentrations in liver tissue and stools.

When triglyceride levels of groups enriched with cholesterol (CAS + COL and COG + COL) were compared, the latter showed significantly lower rates for the parameter, although it was higher than that found in controls ($F = 13.0555$; $p < 0.04$). It is interesting to note that the HDL cholesterol in diets with *A. brasiliensis* is significantly higher than that found in rats with no mushrooms in the diet.

Table 3. Cholesterol levels, triglycerides and HDL Cholesterol of rats fed on Casein Standard Diet, (CAS); Casein Standard Diet plus 1% Cholesterol (CAS + COL); Casein Standard Diet plus *Agaricus* (COG) and Casein Standard Diet plus *Agaricus* and 1% Cholesterol (COG + COL).

Variable		Treatment*			
		CAS	CAS + COL	COG	COG + COL
Total Cholesterol (mg dL^{-1})	IT	103.88 (11.33)[a]	101.12 (10.75)[a]	103.42 (13.11)[a]	102.04 (11.96)[a]
	FT	107.93 (12.14)[a]	142.21 (17.01)[b]	106.29 (10.50)[a]	111.63 (9.11)[a]
	p**		< 0.05		< 0.05
Triglycerides (mg dL^{-1})	IT	71.24 (7.11)[a]	73.48 (8.03)[a]	74.13 (11.14)[a]	73.89 (6.93)[a]
	FT	78.74 (5.43)[b]	106.61 (10.01)[c]	61.49 (6.36)[a]	78.33 (5.82)[b]
	p		< 0.05	< 0.05	
HDL Cholesterol (mg dL^{-1})	IT	45.81 (5.76)[a]	46.17 (4.97)[a]	45.68 (6.05)[a]	46.40 (4.54)[a]
	FT	41.56 (4.28)[a]	37.12 (7.02)[a]	65.34 (8.66)[c]	59.48 (9.07)[c]
	p		< 0.05	< 0.05	< 0.05

*Different letters on each line demonstrate statistically significant differences between treatments at p!<!0.05 level. **IT = Initial Time FT = Final Time ***p value means the result of ANOVA and Tuckey's post-hoc test between IT and FT for the 4 variables. Results in each column represent means and standard deviation.

When the groups fed on diets with the addition of cholesterol (CAS + COL x COG + COL) are compared, it may be observed that the group on diet containing *Agaricus* tends to remove large amount of lipid in stools ($F = 18.5294$ $p < 0.01$). Since this does not occur with added cholesterol group fed on diet with casein, diets with added protein and fiber from the mushroom *A. brasiliensis* lower the effect of deposition of hepatic cholesterol when subjected to an overload of dietary cholesterol intake when compared to control animals fed on casein diet whose source fiber is only cellulose.

Table 4 lists concentration of cholesterol and hepatic lipids and shows that both groups of rats (COG group and COG + COL group) reduced the cholesterol deposited in the liver when compared to controls (CAS and CAS + COL). The above demonstrates that, in spite of the concentration of dietary cholesterol, there is approximately a 32% decrease in the hepatic cholesterol deposition when *A. brasiliensis* was added to the experimental diet.

Table 4. Hepatic Cholesterol Concentration, Total Hepatic Lipid and Total Stools Lipid Concentration of rats fed on different diets, with or without the addition of cholesterol.

Treatment	Hepatic Cholesterol Concentration (mg g^{-1} of liver)	Total Hepatic Lipid Concentration (g 100^{-1} g of sample)	Total Stools Lipid Concentration (g 100^{-1} g of sample)
CAS	37.88 ± 3.70 [b]	25.04 ± 8.24 [a]	10.61 ± 0.61 [a]
CAS + COL	77.14 ± 12.37 [d]	59.22 ± 3.64 [c]	18.44 ± 0.81 [b]
COG	25.89 ± 3.34 [a]	17.06 ± 3.01 [a]	17.86 ± 2.86 [b]
COG + COL	49.05 ± 8.61 [c]	35.72 ± 2.06 [b]	24.50 ± 1.26 [c]

*Different letters in each column demonstrate statistically significant differences at $p < 0.05$ level. Results in each column represent means and standard deviation. **p value is the result of ANOVA and Tukey's post-hoc test between treatments.

When treatments of groups CAS + COL and COG + COL are compared, a significant difference with a decrease in hepatic cholesterol levels of the same magnitude (approximately 36%) may be perceived. However, comparing deposited amount of cholesterol in the liver of rats with different treatments reveals that those fed on mushroom-based diets show a significantly lower concentration ($F = 8.5465$; $p < 0.01$) than controls, regardless of higher oral intake of cholesterol. Results agree with results by Zhang, Li, Liu and Xia (2008) and Martinez-Flores, Chang, Martinez-Bustos, and Sgarbieri (2004), who carried out similar experimental studies using other sources of dietary fiber and reported statistically significant differences ($p < 0.05$) for hepatic cholesterol.

The above data demonstrate that fungus-based diet recovers the deposition of cholesterol in the liver and that *Agaricus* content diet reduces the lipid absorption by excreting it in the stool, regardless of the addition of cholesterol in the food matrix. Therefore, the concentration and quality of dietary fiber component present in the composition of the mushroom *A. brasiliensis* significantly reduce absorption and utilization of important lipid fractions carried by the diet (Hsu, Hwang, Chiang, & Chou, 2008).

The trend of deposition of hepatic cholesterol described above may be applied to the deposition of lipids in the liver. Rise in lipid concentrations in the experimental diets increases lipid concentrations in the liver tissue, regardless of *Agaricus*. A comparison between the treatment groups COG and COG + COL shows that the same effect of lipid deposition in the liver may also be observed in the control group, which could be reproduced.

Further, since there is no significant difference when lipid depositions in the livers of CAS and COG groups are compared, the addition of the mushroom *A. brasiliensis* does not significantly influence levels of lipids on the liver in situations where the amount of lipids in the diet offered are considered normal. However, when different diet standards in which cholesterol was added are compared (CAS + COL and COG + COL), the effect of attenuation appears in the total lipid fraction of mushroom-added diet. This is highly significant ($p < 0.01$) and confirms the effect of non-digested polysaccharides subjected to fermentation in the large intestine of animals.

Table 4 also shows the rates of lipid concentrations in the animals´ stool. Since there is a significant difference in fecal excretion of lipids when CAS and COG groups are compared, concentration of total lipids excreted in the stool

increases when the mushroom *A. brasiliensis* is added to the experimental diet. Similarly, comparing treatment groups and CAS + COL and COG + COL shows a significant difference and observation of virtually the same effect of increased fecal excretion of lipids.

When test and controls are correlated, the elimination of lipids in stool increases linearly ($r = 0.27$; $p = 0.03$; $n = 14$ for CAS and CAS + COL and $r = 0.32$; $p = 0.01$; $n = 14$) with deposition of cholesterol in the liver. The above demonstrates that even if there is no effort for the elimination of metabolites by fecal lipids, lipid overload in the diet frequently causes hepatic deposition.

When the data of metabolic behavior are correlated to the serum lipid profile, a negative correlation occurs between the concentration of triglycerides in the blood and stools ($r = -0.72$; $p = 0.003$; $n = 14$). However, no significant correlation exists between the concentration of lipids in the blood and their deposition on the liver ($r = 0.48$; $p = 0.064$; $n = 14$) after 20 days of consumption of a diet high in cholesterol. Therefore, components from the *Agaricus*-based diet could affect the excretion of lipids and modulate the levels of these macronutrients in an independent way on different compartments of the rat body during their metabolism.

When correlation data of blood glucose and those of lipid profile are analyzed, it has been reported that animals fed on *A. brasiliensis* mushroom maintained high levels of correlation between these markers. Remarkably for the COG group, blood glucose and total cholesterol showed a significant positive correlation ($r = 0.36$; $p = 0.004$; $n = 14$), glucose and triglycerides as well ($r = 0.52$; $p = 0.0001$; $n = 14$), with blood glucose and HDL featuring significant negative correlation ($r = - 0.70$; $p = 0.0001$; $n = 14$). Lower blood glucose (or attenuation of glycemic curve), lower rates of total cholesterol and triglycerides and higher HDL cholesterol rates were registered, corroborated by Yea-Woon, Ki-Hoon, Hyun-Ju, & Dong-Seok (2005).

Table 5 shows the histopathological analysis in rat livers fed on different diets, with or without cholesterol addition. On the other hand, Figure 2 is a photographic record of histological slides. Or rather, rat groups fed on cholesterol-enriched diets have a significant infiltration of fat in the liver and high degree of impairment of the hepatic parenchyma when compared to those of controls. Dilatation cells, displacement from core to periphery and clustering of hypertrophied hepatocytes occur.

Table 5. Histochemical analysis in livers of rats fed on different diets, with or without cholesterol addition.

Tissue	Treatment	Mean area of lesion (%)	p
Fat cells	CAS	18.4687 ± 0.6498 [b]	< 0.001
	CAS + COL	29.3874 ± 0.8932 [c]	
	COG	10.2163 ± 0.7267 [a]	
	COG + COL	19.6421 ± 0.6934 [b]	
Liver parenchyma cells	CAS	86.831 ± 1.5455 [b]	< 0.005
	CAS + COL	72.106 ± 2.284 [a]	
	COG	93.112 ± 1.920 [c]	
	COG + COL	82.417 ± 1.813 [b]	

*Different letters in each column mean statistical significant differences at p! < !0.05 level. The p value is the result of ANOVA and Tukey's post-hoc test between treatments. Results in each column represent means and standard deviation.

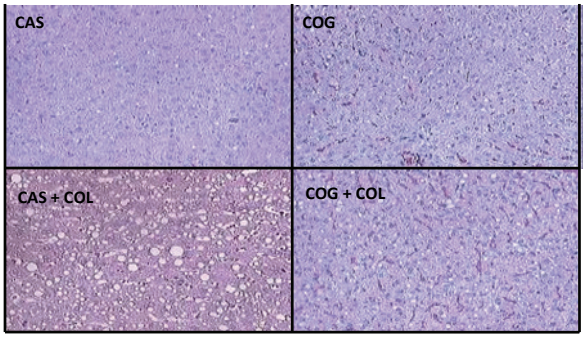

Figure 2. Histochemical analyses of rat liver tissue stained with hematoxylin-eosin in animals fed on Casein Standard Diet, (CAS); Casein Standard Diet plus 1% Cholesterol (CAS + COL); Casein Standard Diet plus *Agaricus* (COG) and Casein Standard Diet plus *Agaricus* and 1% Cholesterol (COG + COL).

In the group supplemented with cholesterol with the addition of *Agaricus*, the amount of fat cells and the percentage of damage to the liver parenchyma are equivalent to those displayed by control rats with casein-based diet ($p < 0.05$). The three consolidated data of the experimental groups analyzed: (i) cholesterol concentration, (ii) total lipid concentration and (iii) histopathological analysis of the livers, clearly demonstrate the effects of *A. brasiliensis* food matrix on the metabolic lipid profile in rats. It should be noted that serum data represent the distribution trend of lipids in the short term (recent profile), while the data from liver tissue reflect the metabolism of medium in the long run, wherein the mobilization of fatty acids takes place followed by the kinetics influenced by the bioavailability of dietary lipids and cross sectional factors, such as intake of dietary fiber.

From a nutritional perspective, *A. brasiliensis* is rich in water, carbohydrates and proteins mainly in dehydrated form, as in current study. It is a source of diet fibers, with low fat contents and a lipid profile in which UFAs predominate more than SFA levels (Grangeia, Heleno, Barros, Martins, & Ferreira, 2011).

Fukushima, Ohashi, Fujiwara, Sonoyama, & Nakano (2001) have demonstrated that serum concentrations of total cholesterol in rats

supplemented with mushrooms decreased from 11 to 25% when compared to control group. Total cholesterol concentrations decreased in the two treatment groups due to reduction of lipoproteins of low and very low density lipoproteins (LDL and VLDL cholesterol). They found no significant differences in liver concentrations of mRNA coding for apo-B between the groups. Another report (Meng, MShen, Zhang, & Sheng, 2012) registered that mRNA level for hepatic LDL receptor in rats fed on the mushroom *Agaricus bisporus* fiber was significantly higher than that of rats fed on cellulose. Further, the level of mRNA for the hepatic LDL receptor was negatively correlated with serum fractions of VLDL + LDL + IDL. The same researchers did not find significant differences in the concentration of cholesterol and hepatic levels of mRNA for HMG-CoA reductase between the experimental groups. Gonçalves, Roma and Gomes-Santos (2012) revealed that *Agaricus stratus* lowered inflammatory stimulus to adipose tissue and had significant impact on risks in atherosclerosis development.

Van Besten et al. (2013) have reported that the products of sugar beet fiber fermentation by cecal bacteria cooperate to lower plasma cholesterol in rats and that SCFA fermentation product may suppress the synthesis of hepatic and intestinal cholesterol in rats. Mascaro et al. (2014) reported that the concentration of cecal propionate stimulates hepatic cholesterol synthesis via high fecal excretion of steroids. They also reported that the concentration of cecal propionate had a negative correlation with plasma cholesterol concentration and positively with cecal neutral steroids and bile acids.

Oh et al. (2010) confirmed in their study the strong capacity of low lipid levels of *Maitake*, an edible macrofungi, similar to *A. brasiliensis*, due to the acceleration on turnover of bile acids and cholesterol and the conversion of cholesterol to bile acids. Further, Cheung (1998) have reported that straw mushroom, consumed in experimental diets, reduced plasma levels and hepatic cholesterol, and increased fecal excretion of neutral sterols in hamsters.

Recent findings on liver tissue of rats fed with *Agaricus*-extracted polysaccharides have demonstrated their ability to modulate the metabolism of the body, both in normal and in lesions caused by virus infection. The lower incidence of lesions correlated strongly with the low fat deposition in the liver of infected animals (Chung-Hua, Kung-Chang, Yi-Hsiung, & Pesus, 2008). Many molecules synthesized by macrofungi, such as polysaccharides, proteins, fats, minerals, glycosides, alkaloids, volatile oils, terpenoids, tocopherols, phenolics, flavonoids, carotenoids, folates, lectins, enzymes and organic acids in general are known to be bioactive, and these compounds are found in different biological parts of edible mushrooms. Polysaccharides are the most important for modern nutrition and medicine, whilst β-glucan is the best known and the most versatile metabolite with a wide spectrum of biological activities (Chang & Wasser, 2012). Studies conducted on rats have demonstrated effective action of the bioactive compounds found in the genus *Agaricus* macrofungi on the liver tissue, especially as protective factors of cell types whose activity is essential for the metabolism of fatty acids and other organic biomolecules (Hsu et al., 2008).

Although no difference has been reported with regard to the weight of rats during the short time spent on the experiments in current study, the facts underscore that diets high in cholesterol induce the redistribution of body fat mass. This may directly influence the lipid profile and glucose, mainly by the development of insulin resistance, an important marker for the development of Type 2 diabetes (Vasques et al., 2007). Data in current study indicate an improvement trend in these behaviors when the diet contains significant amounts of dietary fiber derived the matrix *A. brasiliensis*.

Conclusion

Based on the results in current study the following conclusions may be drawn:

The nutrients in *A. brasiliensis* are important modulators of the lipid profile of Wistar rats, significantly reducing cholesterol, triglycerides and lipid deposition on liver tissue and increasing their removal by stool. Glycemic behavior showed that rats fed on mushrooms had significant attenuation on glucose curve for up to sixty minutes, contributing to a minor variation in blood glucose levels.

Diets prepared with macrofungi also proved to be able to exert a dampening effect upon the addition of exogenous cholesterol, contributing significantly to the maintenance of acceptable levels of metabolic risk parameters analyzed and reinforcing the need for intake of dietary fiber sources in the context of a mixed diet as an important protective factor against the development of chronic diseases (NCDs), such as dyslipidemias, diabetes and cardiovascular diseases.

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