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ORIGINAL CONTRIBUTION

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A study on the nutritive value of pollen from the Chinese Masson Pine (Pinus massoniana) and its effect on fecal characteristics in rats

Untersuchungen zum Nährwert von Pollen der chinesischen Masson Pinie (Pinus massoniana) und dessen Effekt auf die Zusammensetzung des Kots bei der Ratte

Summary A digestion experiment with growing rats was conducted to study the effect of native and broken pollen of Chinese Masson Pine (*Pinus massoniana*) on fecal composition and digestibilities of dry matter, crude protein and crude ash. For that issue each 10 of 30 animals were fed a semisynthetic diet containing either no pollen or an addition of 6 % native or broken pollen for 17 days at restricted amounts. In a second experiment each 6 of 12 growing rats received a semisynthetic diet containing either no or 5 % broken pollen. At day 4, 11 and 18 samples of the feces were collected and analyzed for mesophilic aerobic bacteria. The pollen contained large amounts of cell wall constituents (26–30 % lignin and 10–15 % cellulose, 2–13 % hemicellulose) with higher values found in native pollen. The contents of crude protein, crude fat, total lipids, available carbohydrates (starch and sugars), crude ash, gross energy and metabolizable energy were 13 %, 2–10 %, 8–10 %, 17–18 %, 3.1–3.5 %, 21–22 kJ/g and 5.7–9.0 kJ/g. The addition of native pollen to the diet increased the total amount of feces by 71 % as compared to the control level. The fecal contents of dry matter and of crude protein and crude ash in fecal dry matter changed by -5, +4 and -5 percentage units. The apparent digestibilities of dry matter and crude protein decreased by 3 and 5 percentage units, respectively, while the apparent digestibility of crude ash remained unchanged. Broken pollen acted in the same direction, however the effects were about 20 % less pronounced as compared to native pollen. The pollen feeding reduced the fecal germ contents of *Proteus mirabilis* and *Escherichia coli*, while the amounts of α-hemolysing streptococci was increased. In total, the observed effects of an addition of native broken or pollen to the diet seemed to be based mainly on the increased intake of cell wall constituents.

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Zusammenfassung In einem Verdauungsversuch mit wachsenden Ratten wurde die Wirkung von nativem oder gemahlenem Pollen der chinesischen Masson Pinie (*Pinus massoniana*) auf die Zusammensetzung des Kots und die Verdaulichkeit von Trockenmasse, Rohprotein und Rohasche geprüft. Dazu erhielten je 10 von 30 Tieren 17 Tage lang restriktiv eine semisynthetische Diät entweder ohne Pollen oder mit einem Zusatz von 6 % nativen oder gemahlenen Pollen. In einem weiteren Experiment wurde an je 6 von 12 wachsenden Ratten eine semisynthetische Diät verfüttert, die entweder keine oder 5 % gemahlene Pollen enthielt. Nach 4, 11 und 18 Tagen wurden Kotproben gezogen und deren Gehalte an mesophilen aeroben Bakterien analysiert. Die Pollen enthielten hohe Mengen an Gerüstsubstanzen (26–30 % Lignin und 10–15 % Cellulose, 2–13 % Hemicellulose), wobei die nativen Pollen die jeweils höheren Werte aufwiesen. Die Gehalte an Rohprotein, Rohfett, Gesamtfett, verwertbaren Kohlenhydraten (Stärke und Zucker), Rohasche sowie Brutto- und umsetzbarer Energie betrugen 13 %, 2–10 %, 8–10 %, 17–18 %, 3,1–3,5 %, 21–22 kJ/g bzw. 5,7–9,0 kJ/g. Der Zusatz nativer Pollen zur Diät erhöhte die fäkale Ausscheidung an Frischmas-

se um 71 % gegenüber der Kontrollgruppe. Der Trockenmassegehalt des Kots und die Konzentrationen an Rohprotein und Rohasche in der Kottrockenmasse veränderten sich gegenüber dem Kontrollniveau um -5, +4 und -5 Prozentpunkte. Die scheinbaren Verdaulichkeiten der Trockenmasse und des Rohproteins sanken um 3 bzw. 5 Prozentpunkte, während die scheinbaren Verdaulichkeiten der Rohasche unverändert blieben. Gemahlene Pollen hatten die gleiche Wirkungsrichtung wie native Pollen, der Gesamteffekt war jedoch im Mittel um etwa 20 % schwächer ausgeprägt. Die Verfütterung von Pollen reduzierte die fäkalen Keimzahlen von *Proteus mirabilis* und *Escherichia coli*, während die Gehalte an α-hämolysierenden Streptokokken zunahm. Insgesamt dürften die beobachteten Effekte des Zusatzes von nativen und gemahlenen Pollen zur Diät weitgehend auf die erhöhte Zufuhr von pflanzlichen Gerüstsubstanzen zurückzuführen sein.

Key words Pollen – Chinese Masson Pine – nutritive value – feces – digestibility

Schlüsselwörter Pollen – Chinesische Masson Pinie – Nährwert – Fäzes – Verdaulichkeit

Introduction

The pollen from the Chinese Masson Pine (*Pinus massoniana*) is a medicinal herb of the traditional Chinese medicine. Its use was described already in the earliest Chinese pharmacopoeias written during the Tang and Ming dynasty (14, 11). One of the main fields of the traditional application of Masson Pine pollen is the care of chronic constipation especially in elder men (20). According to the concept of the traditional Chinese medicine the Masson Pine pollen is used as a food additive and its application to men may have not only possible pharmacological but also nutritional effects. Moreover, recent advances in collection, storage and processing techniques made Masson Pine pollen available for the food and cosmetic industry (22). Therefore, the present study was designed to characterize the nutritional aspects of Masson Pine pollen with special emphasis on nutrient contents and the effects on fecal characteristics and digestion parameters. Since recently a new method of breaking the pollen was established, the study should include also the comparison between the traditional native and the broken preparation of Chinese Masson Pine pollen.

Material and methods

The pollen used in the present study was derived from the Pine Pollen Development Station of SFRICAF (P.R. China). A part of the pollen was broken by high speed airflow at 6 °C (YanNai New Era Natural Nutrients Ltd. Corp.; P.R. China). In the first part of the present study the native and broken pollen were analyzed *in vitro* for nutritional parameters according to standard procedures. In the second part, the nutritive value of the pollen and its effect on fecal characteristics was tested *in vivo* by a digestion experiment with rats. Additionally, a feeding experiment with rats was conducted to examine the influence of pollen on the fecal microflora.

Analysis of nutritional parameters

The native and broken pollen were analyzed for contents of dry matter, crude protein (N*6.25), crude fat (ether extract), total lipids, crude ash, starch and sugars according to standard procedures (12). The detergent analysis (5) provided the contents of neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin (ADL) of which hemicellulose and cellulose was calculated by subtraction (NDF-ADF and ADF-ADL). The concentrations of zinc were measured by AAS after dry ashing. Soaking the pollen in dest. H_2O at room temperature for 24 h and analyzing the filtrated water for zinc provided the proportion of water-soluble zinc. The true absorbability of pollen zinc was recorded in a metabolism experiment with ^{65}Zn-labeled rats using the isotope dilution technique (16). Gross energy was measured by combustion in a bomb calorimeter while the metabolizable energy was estimated by the nutrient contents (13). In order to describe the hygienic status of the pollen material, its germ content and antibiotic activity was analyzed according to (4, 3).

Digestion experiment

30 growing male Sprague Dawley rats were housed in metabolic cages at standardized environmental conditions (23 °C; relative humidity of 60 %; light/dark cycle of 12 h). At a mean body weight of 85 g the animals were divided into 3 groups of 10 animals each and fed diets containing either no pollen, or 6 % pollen in the native or broken preparation. The purified semisynthetic control diet consisted of 20 % casein, 33 % starch and 30 % saccharose, 8 % fat, 3 % cellulose and 6 % mineral and vitamin mixture. The pollen diets were provided by adding the pollen to the control diet at rates of 6 %. The animals were fed the respective diets for 17 days at restricted amounts (9.4 g/day). The fecal excretions of day 11 to 17 were collected quantitatively and stored in airtight vessels at 4 °C. At the end of the collection

period the feces were pooled within each animal and analyzed for fresh weight, dry matter, crude protein and crude ash (12). The determination of crude protein was performed in fresh feces.

Examination of the fecal microflora

12 growing female Sprague Dawley rats with an initial body weight of 150 g were housed individually at standardized environmental conditions (23 °C; relative humidity of 60 %; light/dark cycle of 12 h). The animals were divided into 2 groups (n = 6) and were pairfed a diet containing either no pollen or 5 % broken pollen for 18 days. The composition of the diets was almost identical to that used in the preceding digestion experiment. At day 4, 11 and 18, samples of feces were collected and pooled within the two dietary groups. Immediately afterwards the samples were analyzed for contents of aerobic mesophilic germs according to (4) with modifications (suspension of feces in pepton water at a ratio of 1:9, incubation at room temperature for 20 minutes, inoculating on blood agar, nutrient agar and Gassner agar, incubation at 37 °C for 48 h).

Statistics

The results of the *in vivo* studies are presented as mean values of the treatment groups together with the standard deviation of the single values. The means of the digestion experiment were tested for differences by a multiple comparison procedure (Student-Newman-Keuls test) after submitting the data to an oneway analysis of variance with the type of diet as the influencing factor. The means of the fecal germ contents of the two treatment groups were tested for differences by a paired *t*-test (within collection day). Mean values with no common superscript are significantly different ($p < 0.05$).

Results

Figure 1 shows the exterior of native and broken Masson Pine pollen as retrieved from electronic microscopy. The size and shape was typical for pollen from conifers. The whole particles were about 40 µm in diameter and consisted of a main part covering two *sacci*, which serve as airbags for the anemogamic pines. The inner structure especially for the *sacci* was like a honeycomb as can be seen in the case of broken pollen.

Table 1 presents the nutrient composition of native and broken pollen. In native pollen the contents of crude protein, crude fat and total lipids were 12.7 %, 1.5 % and 7.3 % of fresh matter. The pollen material consisted for 56.8 % of cell wall constituents (hemicellulose, cellulose, lignin) with lignin as the dominating component. On the other hand, available carbohydrates (starch and sugars) accounted for only 17.7 %. Crude ash and total zinc was 3.1 % and 37 ppm. The zinc was highly absorbable (91 %) despite the low water solubility (0.5 %). The gross energy of native pollen was 21.0 kJ/g of which 5.7 kJ/g were metabolizable. Broken pollen did not differ substantially from native pollen with respect to the contents of crude protein (13.0 %), available carbohydrates (17.7 %) and gross energy (22.1 kJ/g). However, crude fat was higher (10.5 %) and cell wall constituents were lower (44.0 % in total) as compared to native pollen. Also the true absorbability of zinc and the metabolizable energy changed to higher levels (96 % and 9.0 kJ/g).

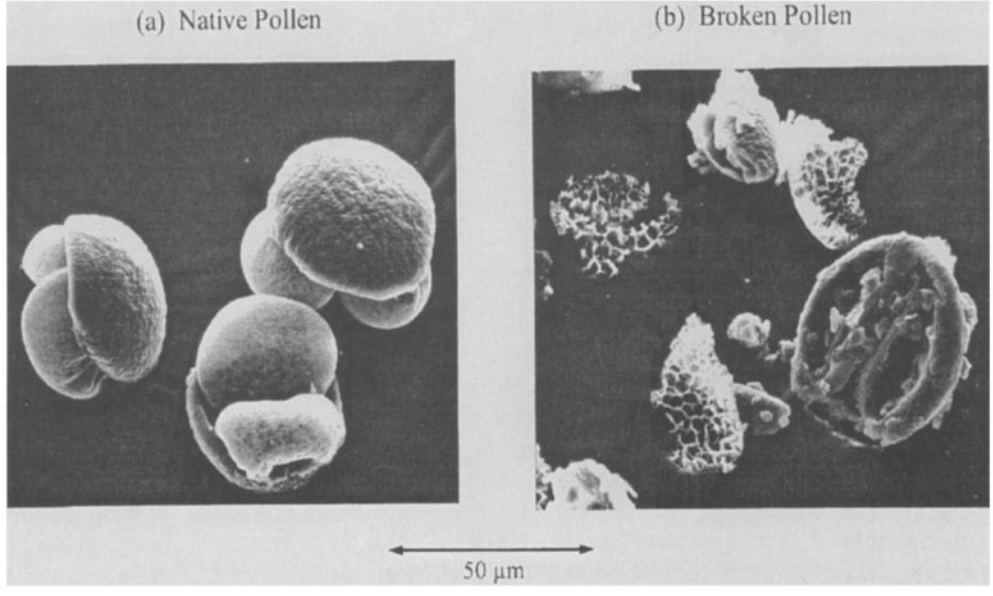

Fig. 1 Electronic microscopy of Masson Pine pollen: (a) Native pollen; (b) Broken pollen

Table 1 Nutritional parameters of native and broken pollen

	Native pollen	Broken pollen
Dry matter (%)	94.7	94.1
Nutrients (% of fresh matter)		
Crude protein	12.7	13.1
Crude fat	1.5	10.5
Total lipids	7.3	10.0
Crude fiber	35.6	27.3
Hemicellulose	12.8	1.5
Cellulose	14.4	9.9
Lignin	29.6	25.9
Starch	5.4	7.0
Sugars	12.3	10.0
Crude ash	3.1	3.5
Zn, total (µg/g)	36.6	37.0
Zn, water soluble (% of total Zn)	0.4	0.7
Zn, true absorbable (% of total Zn)	91	96
Energy (kJ/g)		
Gross energy	21.0	22.1
Metabolizable energy	5.7	9.0
Germ contents ($CFU/_{lg}$)[1]		
Total mesophilic bacteria (mainly Bacillus spp.)	4.5	3.9
Enterobacteriaceae	< 2.0	< 2.0
Yeasts	< 2.0	< 2.0
Moulds (Aspergillus niger and Mucoraceae)	2.3	2.3
Antibiotic activity (equivalent to Penicillin G, µg/g)	< 0.02	< 0.02

[1] $\log_{10}$ of colony forming units per g of sample

With respect to the hygienic status, both native and broken pollen revealed low germ contents of 4 CFU_{lg}/g for mesophilic bacteria, < 2 CFU_{lg}/g for enterobacteriaceae and yeasts and 2 CFU_{lg}/g for moulds. The antibiotic activity per g of native and broken pollen was less than an equivalent of 0.02 µg Penicillin G.

As shown in Table 2, the animals fed the control diet excreted 0.49 g fresh feces per day. However, with native and broken pollen in the diet the fecal mass increased to 0.84 and 0.81 g/d. Simultaneously, the dry matter content of the feces decreased from 88.0 % (control) to 82.3 % (native pollen) and 81.7 % (broken pollen). This effect compensated only partially the higher excretion of fresh matter. Thus, the digestibility of dry matter fell from 95.1 % (control) to 91.1 % (native pollen) and 92.5 % (broken pollen). The control level of the fecal content and the apparent digestibility of crude protein was 13.0 % and 95.8 %, respectively. This parameters changed to 16.9 % and 91.1 % in the native pollen group. With broken pollen the fecal content and apparent digestibility of crude protein shifted in the same direction (14.8 % and 92.5 %), however, the effect was less pronounced as compared to native pollen. The contents of crude ash in fecal dry matter were reduced from 13.2 % (control) to 8.1 % (native pollen) and 8.0 % (broken pollen) while the apparent digestibility of crude ash averaged 84.2 % irrespective of treatment.

Table 3 presents the contents of aerobic mesophilic germs in the feces. In animals fed the control diet the fecal pattern of aerobic mesophilic germs consisted mostly of *Proteus mirabilis, Escherichia coli* and α-hemolysing streptococci at amounts of 6.6, 6.8 and 6.7 CFU_{lg} per g fresh feces. Feeding broken pollen reduced the fecal contents of *Proteus mirabilis* and *Escherichia coli* to 6.0 and 6.2 CFU_{lg}/g while in α-hemolysing streptococci an increase to 6.7 CFU_{lg}/g was observed. This shift in fecal germ pattern was statistically significant in the case of *Escherichia coli* and α-hemolysing streptococci.

Discussion

The nutrient composition of Masson Pine pollen was characterized in general by large amounts of cell wall components. On the other hand, the content of available carbohydrates was low and the proportions of protein and fat were not pronounced as compared to other food from plant origin. Consequently, the Masson Pine pollen contained only little metabolizable energy. Some enhancements of the nutritive value could be observed in broken pollen. The nutrients imbedded into the pollen seemed to be more available in broken than in native pollen as can be seen in the higher amount of analyzed crude fat. On the other hand, the reduction of cell wall constituents indicated losses of fiber rich particles during the breaking procedure (according to microscopical judgment the losses consisted mainly of the *sacci* of the pollen). In total, the differences between native and broken pollen were of minor importance in the face of the overall low nutrient concentration. Also in relation to honeybee-collected pollen the nutritional value of Masson Pine pollen ranged at low levels (1, 2). However, the composition of honeybee-collected pollen may vary over a wide range mainly depending on the vegetation around the beehouses (2). On the contrary, Masson Pine pollen is harvested directly by man and is available in a pure form. This may be favorable also to the hygienic stability of the pollen products. As can be seen at the low contents of mesophilic bacteria, yeasts and moulds there was no complaint about the hygienic status of both native or broken pollen.

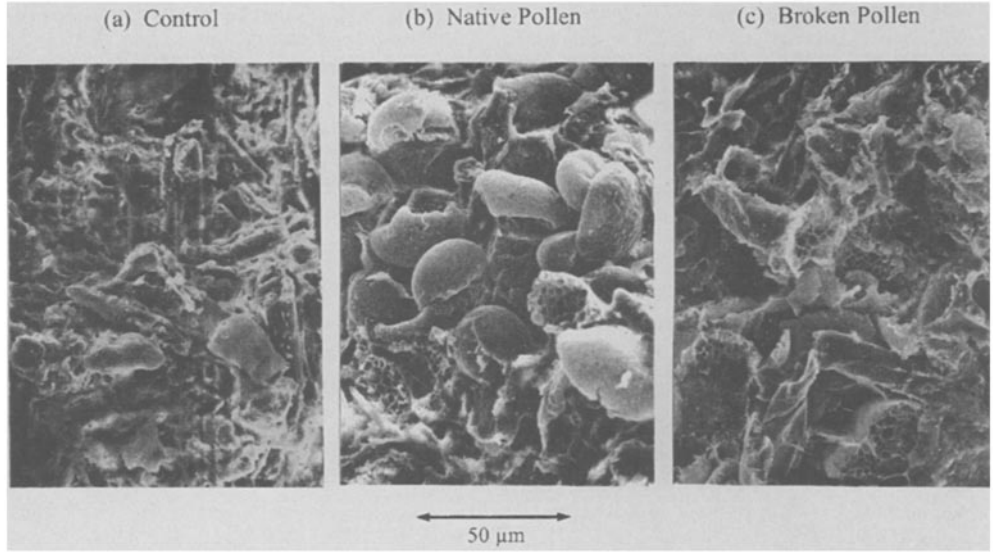

Fig. 2 Electronic microscopy of feces: (a) Control; (b) Native pollen; (c) Broken pollen

Masson Pine pollen reduced significantly the dry matter digestibility of the complete diet. Taking into account that 6 % of total dry matter consumption resulted from pollen, the digestibility of pollen dry matter was 43 % and 50 % in native and broken pollen, respectively. These low levels of digestibility corresponded to the high fiber contents of the pollen. Cellulose for example can be degraded during the passage through the intestinal tract of monogastric species only at about half of the ingested amounts (9, 10). Lignin is almost indigestible and it may additionally impair the intestinal degradation of other nutrients (7). The depressive effect of dietary fiber on dry matter digestibility is reflected also in the lower digestion coefficient in animals fed native pollen as compared to the broken pollen group. The large amounts of poorly digested pollen material can be seen as well by microscopy of the feces as shown in figure 2.

The fecal dry matter content was significantly reduced in animals fed native or broken pollen. This observation may be explained mainly by the higher intake of dietary fiber. As known widely from digestion experiments with monogastric species including humans, the consumption of fiber rich food increases the fecal water content, especially if the fiber is well degradable by the hindgut microorganisms (7, 15, 17, 18). The latter may explain why the lower fiber content of broken as compared to native pollen did not affect the fecal water concentration. Obviously, the change in fiber composition of broken pollen was accompanied by an enhanced fiber degradability. Besides that effects of dietary fiber on fecal water content, the large surface of the honeycomb-structured pollen particles may have exerted an additional water binding effect.

The combination of the lower dry matter digestibility with the higher fecal water content resulted in a substan-

Table 2 Fecal characteristics and apparent absorption of fresh and dry matter, crude protein and crude ash

	Control diet	Native pollen	Broken pollen
Fresh matter:			
Excretion with feces (g/day)	$0.489^{b} \pm 0.066$	$0.841^{a} \pm 0.117$	$0.815^{a} \pm 0.109$
Dry matter:			
Content in feces (%)	$88.0^{a} \pm 3.7$	$82.3^{b} \pm 4.6$	$81.7^{b} \pm 5.8$
Apparent digestibility (% of intake)	$95.1^{a} \pm 0.4$	$92.0^{c} \pm 0.2$	$92.4^{b} \pm 0.7$
Crude protein:			
Content in feces (% in dry matter)	$13.0^{c} \pm 3.3$	$16.9^{a} \pm 2.1$	$14.8^{b} \pm 4.1$
Apparent digestibility (% of intake)	$95.8^{a} \pm 1.2$	$91.1^{c} \pm 1.2$	$92.5^{b} \pm 2.2$
Crude ash:			
Content in feces (% in dry matter)	$13.2^{a} \pm 2.0$	$8.1^{b} \pm 0.7$	$8.0^{b} \pm 0.9$
Apparent digestibility (% of intake)	84.3 ± 3.0	83.7 ± 1.8	84.5 ± 2.3

Table 3 Contents of aerobic mesophilic germs in the feces (CFU_{lg}/g)[1)]

	Control	Broken pollen
Proteus mirabilis	6.8 ± 0.8	6.0 ± 1.5
Escherichia coli	6.8[a] ± 0.9	6.2[b] ± 0.9
α-hemolysing streptococci	6.7[b] ± 0.7	7.3[a] ± 0.6

[1)] $\log_{10}$ of colony forming units per g of sample

tial increase of the daily amount of fresh feces by about two thirds as compared to the control level. This fecal bulking effect seems to correspond to the traditional Chinese medicine which recommends Masson Pine pollen as a food additive in the care of chronic constipation in men (11, 14, 20). However, the extent of the pollen effect has to be interpreted also in connection with the control situation. Since the high digestibility of the control diet caused only little amounts of feces with low water contents, the fecal bulking effect of the pollen appeared particularly pronounced. Mathematically, the intake of 1 g pollen increased the fecal bulk by 0.6 g on an average. Similar effects can be observed also with other fiber rich food as carrots for example if differences in dry matter content are taken into account (17, 18).

The addition of pollen to the diet increased the fecal protein concentration and reduced the apparent digestibility of dietary protein. However, this effect is not to be interpreted as an indicator of a depressed intestinal availability of dietary amino acids. Since fecal protein is mainly of bacterial origin, it reflects the extent of the hindgut fermentation (6, 9, 10, 19). Therefore, the increased fecal protein concentration in animals fed pollen resulted from the higher fiber intake which provided additional amounts of bacterially fermentable substrates to the hindgut microflora. This mechanism explains also the lower effect of broken pollen on fecal protein concentration as compared to native pollen, since in broken pollen the overall content of fiber was reduced.

The Masson Pine pollen reduced the fecal contents of *Proteus mirabilis* and *Escherichia coli* while α-hemolysing streptococci were found at higher amounts. This shift in microbial pattern should be beneficial for the organism, especially with respect to the suppression of the facultative pathogen germs *Proteus mirabilis* and *Escherichia coli*. However, no relevant antibiotic activity could be attributed to the pollen. Obviously, the differences in fecal germ contents were based mainly on changes in dietary composition as for example the increased supply of bacterially fermentable substrates to the hindgut microflora. Also an accelerated passage rate of the gut fill due to the higher dietary fiber content (8) may have contributed to the observed shift of the fecal microbial pattern.

The mineral content of Masson Pine pollen as estimated by crude ash was comparable to other food of plant origin and also to honeybee-collected pollen (1, 2). The zinc of the Masson Pine pollen revealed a high potential for true absorbability ranging between 91 % and 96 % in native and broken pollen, respectively (16). However, the total zinc content of pollen was not unusually high. Moreover, pollen is consumed only in low quantities and therefore it might not contribute significantly to zinc supply in human nutrition. Nevertheless, the high zinc absorbability indicates that Masson Pine pollen does not contain relevant amounts of mineral complexing agents like phytate and polyphenols. This may be concluded also from the apparent digestibility of the dietary crude ash which did not differ among the three treatment groups of the digestion study.

In conclusion, the Masson Pine pollen cannot be classified as an important source of macronutrients, energy or minerals to human nutrition. On the other hand, the available information about Masson Pine pollen does not point to quantitatively relevant antinutritive factors (16, 21). Thus, the long term use of Masson Pine pollen as a food additive seems to implement no serious risks of nutritional disorders. Obviously, one main component of the beneficial effects which are attributed to the Masson Pine pollen by the traditional Chinese medicine is based on its high fiber content.

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