Nutritive value of Masson Pine pollen *(Pinus massoniana)* in comparison to wheat bran and effects on stool characteristics in a pig model

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Nutritive Wirksamkeit von Pollen der Massonpinie *(Pinus massoniana)* im Vergleich zu Weizenkleie sowie Auswirkungen auf Faeceschara cteristika beim Modelltier Schwein

1 Introduction

The use of pollen from the Masson Pine (*Pinus massoniana*) in the traditional Chinese medicine has a long history and its medical application was already mentioned in the earliest Chinese pharmacopeias written during the Tang and Ming dynasty (LI, 1978; SU, 1957). Up to now, one of the main application fields of the artificially sampled pollen is the cure of chronic constipation, especially in older men (ZHANG, 1993). However, in recent years, there were some rapid advances in collection, storage and processing techniques and therefore availability of Pine pollen is not longer restricted to medical use but it is also available for the food and cosmetic industry. Despite the long tradition of medical use of Pine pollen in China, there is a lack of experimental data describing the physiological basis on which the

Zusammenfassung

Um den Effekt von Pollen der chinesischen Masson Pinie (*Pinus massoniana*) auf Stuhlvolumen, Faeceschara cteristica und Nährstoffverdaulichkeit zu überprüfen, wurden insgesamt 18 Bilanzmessungen an 9 Schweinen (40–60 kg LM) durchgeführt. Die Tiere wurden in 3 Gruppen aufgeteilt und entweder über eine Basaldiät oder diese Diät, ergänzt mit 3,5 % Pinienpollen oder Weizenkleie, versorgt. Die Pinienpollen erhöhten ($p < 0.05$) das Stuhlvolumen, den Wassergehalt der Faeces und die tägliche Ausscheidung an N, Triacylglyceriden und Cholesterol. Die tägliche Gallensäureausscheidung war im Vergleich zur Weizenkleiegruppe erhöht ($p < 0.05$). Durch die Pollenzulage war die Verdaulichkeit der T, des XP und des XL erniedrigt ($p < 0.05$), die Ausscheidung an verschiedenen Bakterienspezies hingegen erhöht ($p < 0.05$). Insgesamt stellen die Pinienpollen ein geeignetes Additiv zur Verbesserung der Faserversorgung insbesondere in der Humanernährung dar.

Schlüsselworte: Masson Pinenpollen, Faser, Faeceschara cteristica, Bakterien, Modelltier Schwein.

Summary

The effect of pollen of Chinese Masson Pine (*Pinus massoniana*) on stool weight, faecal characteristics and nutrient digestibility was investigated in a total of 18 balance measurements. 9 young pigs (40–60 kg of BW) were subdivided in a randomized latin square to three treatments offered either a control diet, this diet added by 3.5 % of Masson Pine pollen or 3.5 % wheat bran. Masson Pine pollen increased ($p < 0.05$) daily bulk volume, water content of stool and daily faecal nitrogen, triacyglycerol and cholesterol excretion. Daily faecal bile acid excretion was increased ($p < 0.05$) compared to the wheat bran group. Digestibility of dry matter, crude protein and crude lipids was decreased ($p < 0.05$) and daily faecal excretion of different bacterial species was increased ($p < 0.05$) in pollen fed animals. In conclusion, Masson Pine pollen may be a useful additive to elevate fibre supply in men.

Key words: Masson Pine pollen, fibre, faecal characteristics, microbes, pig model.

Masson Pine pollen may act, and this is particularly true for data available to the Western hemisphere. A former study (ZHAO et al., 1996) indicated that the Masson Pine pollen may have some prebiotic effects in rats and this may be attributed to its fermentable fibre fraction. There was some evidence that inclusion of Pine pollen in a rat diet may affect faecal dry matter, crude protein and crude ash concentration as well as nutrient digestibility. Given that those data implicate the use of Masson Pine pollen as a feed additive that may help to enhance dietary fibre supply, in the present study a pig model was used to investigate in detail the nutritive effects of the Chinese Masson Pine pollen and of wheat bran, both being representatives of food additives rich in fibre, in comparison to a poor fibre diet. In contrary to wheat bran, however, the fibre fraction of Masson Pine pollen is particularly rich in insoluble fibre.

2 Materials and Methods

The supplements Masson Pine pollen and wheat bran were compared against a basal diet low in fibre and high in nutrient density containing mainly corn starch, sugar, soybean protein isolate and small amounts of purified cellulose (Table 1, control diet). The test diets (pollen diet and wheat bran diet), were produced by mixing the supplements into the control diet at portions of 3.5 % each.

The Masson Pine pollen was derived from the Pine Pollen Development Station of SFRICAF (P.R. China). Nutrient analysis confirmed a high content of crude fibre of almost 40% in dry matter as observed already in a previous experiment (ZHAO et al., 1996). The wheat bran used in the present study was derived from a commercial food shop (Demeter Weizenspeisekleie, Spielberger KG, Burgermühle, Germany). Crude fibre analysis resulted in a relatively low content of 9 % in dry matter compared to Pine pollen. However, the additions of 3.5 % pine pollen or wheat bran changed the complete feed only to a small extent, and crude nutrient contents were quite similar among the three experimental diets.

The diets were tested in an animal model employing a total of nine male-castrated, growing pigs (crossbred of Pietrain and German Landrace) weighing 36 kg at the begin of the study. The three diets were randomly distributed to the animals. The feed was offered restrictively according to the following schedule: the individual body weight at the start of the

Table 1: Composition of diets and supplements
Tabelle 1: Zusammensetzung der Rationen, des Pinienpollens und der Weizenkleie

	Basal diet (control)	Pine pollen diet	Wheat bran diet	Pure Pine pollen	Pure wheat bran
Ingredients (g/kg DM)					
Corn starch	500				
Sucrose	207				
Soybean-protein isolate	200				
Soybean oil	30				
Cellulose	30	+ 35 g	+ 35 g		
Mineral and Vitamin mixture*	20	Pine pollen	Wheat bran		
$CaHPO_4$ (40 % P)	8.4				
DL-methionine	2.1				
L-lysine · HCl	1.5				
L-threonine	1.0				
L-tryptophan	0.04				
Nutrient analysis (g/kg DM)					
Crude protein	187	189	184	116	122
Crude fibre	27	38	30	387	90
Total lipids	39	40	40	93	50
N-free extracts	708	695	710	367	684
Crude ash	39	38	36	37	54

* Per kg of mineral and vitamin mixture: P, 9.4 %; Ca, 21 %; Na, 6 %; Mg, 1.5 %; retinol, 600,000 IE; cholecalciferol, 66,000 IE; α-tocopherol, 2,000 mg; menadione, 80 mg; thiamin, 80 mg; riboflavin, 120 mg; pyridoxine, 100 mg; cyanocobalamin, 100 µg; pantothenic acid, 270 mg; nicotinic acid, 625 mg; biotin, 8,000 µg; choline chloride, 10,000 mg; Zn, 6,000 mg; Cu, 1,000 mg; Fe, 5,400 mg; I, 72 mg; Mn, 3,320 mg, Se, 12 mg

study was extrapolated to the middle of the next 14 d by assuming a growth rate of 0.25 kg/day. This body weight was used to calculate the individual amounts of feed necessary for an unique daily energy intake of 1.1 MJ metabolizable energy (ME) per kg of metabolic body weight ($BW^{0.75}$). The feed was offered twice daily in equal portions after soaking with the 2.75-fold quantity of water. At the end of the 2 weeks feeding period, each animal was weighed, redistributed randomly to another type of diet, and fed restrictively according to its extrapolated body weight for the next 14 d feeding period. By this way, the experiment comprised a total of six replications per dietary group. Two data records had to be excluded from evaluation due to stress-induced mild diarrhoea (1 record from control diet and 1 record from wheat bran diet, each during the first feeding cycle).

The animals were housed individually in metabolic cages. Animal housing and care was conducted under supervision of the veterinarian office of the Bavarian government. The handling protocol ensured proper care and treatment of all animals in conformity with the German law for animal protection. Faeces and urine was collected twice daily over the second week of the 14-d feeding period. Whole faeces and an aliquot (10 %) of urine acidified to pH ~ 3 were stored at 4 °C until the end of each collection period. The faeces were homogenized, subsamples of fresh faeces were derived for further analysis, and the residuals were freeze-dried. Nitrogen contents (Kjehldahl) were determined in feed, fresh faeces, and urine. Compositions of nitrogen in fresh faeces (bacterial – endogenous – undigested dietary protein) were derived according to KREUZER et al. (1991) on the basis of the method of MASON (1981). The diets and the freeze-dried faeces were analyzed for crude fibre, total lipids, N-free extracts and crude ash (NAUMANN and BASSLER, 1983). For the determination of lipids in freeze-dried faeces, lipids were extracted with a mixture of hexane and isopropanol (3:2, v/v, according to HARA and RADIN (1978)). Concentrations of total cholesterol, triacylglycerols and bile acids were determined by commercially available kit reagents (Merck, Darmstadt, Germany) after the lipids of the extracts were suspended in the aqueous solution with Triton X-100 (EDER and KIRCHGESSSNER, 1994). The concentrations of individual fatty acids of faeces were determined by capillary gas chromatography. Lipids from the extract were transmethylated with trimethyl sulfonium hydroxide. Fatty acid methyl esters were separated on a polar capillary column (30 m FFAP, 0.53 mm I.D., Macherey and Nagel, Düren, Germany) with helium as carrier gas. Fatty acid methyl esters were identified by comparing their retention times with those of individual purified standards and quantified using pentadecanoic acid methyl ester as an internal standard. Concentrations of tocopherols in freeze-dried faeces were determined by high-performance liquid chromatography (HPLC, HP 1100, Hewlett Packard GmbH) (BALZ et al., 1993). Samples were saponified with sodium hydroxide in the presence of pyrogallol as an antioxidant for 30 min at 70 °C. The tocopherols were extracted with n-hexane. The tocopherol isomers were separated on a LiChrosorb Si-60 column (5 µm particle size, 250 mm length, 4 mm internal diameter, Merck) using a mixture of n-hexane and 1,4 dioxane (96:4, v/v) as eluent and detected by fluorescence (excitation: 330 nm, emission: 295 nm).

At the end of each feeding period, a sample of fresh faeces was taken directly from the rectum of each animal and immediately stored at –20 °C in an evacuated plastic bag until analysis of bacterial counts. Bacterial counts in faecal samples were determined by the following culture media: sheep-blood agar for *Enterococci* and *Clostridium perfringens*, Gassner-agar for *Escherichia coli*, MRS-agar for *lactic acid* bacteria and TYP-agar as well as tomato agar (Chinese formulation) for *Bifidobacterium spp.*

The data were submitted to an analysis of variance (GLM-procedure of SAS, SAS Inst., Inc., Cary, NC) using the type of diet, the feeding cycle and the respective interactions as factors. The following tables present the mean values of the three experimental diets and the standard deviation (S.D.) as derived from the analysis of variance. Significant differences among means ($p < 0.05$; Student-Newman-Keuls-test) are indicated by superscripts.

3 Results

The mean body weight (data not shown) of the pigs at the start of the second experimental cycle and at the end of the experiment of 43.0 ± 4.0 kg and 50.5 ± 5.1 kg, respectively, showed no difference between treatments. Daily gains over the whole experiment were 533 ± 109 g with no differences between treatment means.

According to the experimental design (restrictive feeding), the mean average daily feed intake of 1127 ± 77 g DM/d showed only a slight variation between the three treatment groups (Table 2). Despite this fact, the 3.5 % pollen additive intensively increased the amount of excreted faeces. On a fresh weight basis, amounts of faeces excreted daily by the pigs of the pollen group were about 98 and 88 percentage points higher ($p < 0.05$) than in the con-

trol and the wheat bran group, respectively (Table 2). Given that DM content of faeces of the pigs in the pollen group was about 9 percentage points decreased ($p < 0.05$), differences in the amount of daily excreted faeces expressed on a DM basis became slightly lower, but, however, remained remarkably ($p < 0.05$).

The faecal crude fibre content in the pollen group of 26.2 % was about 3.5 and 4.6 percentage points higher ($p < 0.05$) than in the control and the wheat bran groups, respectively. The faecal NfE content increased ($p < 0.05$) from 15.6% in the control group to 20.0 and 23.1 % in the pollen and the wheat bran group, respectively. On the contrary, faecal crude ash content was decreased ($p < 0.05$) by 9.0 and 7.8 percentage points, respectively, due to the inclusion of the Pine pollen or wheat bran to the diets. Mean crude protein and crude lipid content of faeces were 19.4 ± 3.0 and 10.7 ± 1.5 %, respectively, and showed no differences between the treatments. However, because of the differences in faeces weight between the dietary treatments, the daily excretion of crude lipid and crude protein (N) of the pollen group was increased compared to the other two groups.

The different fractions of faecal N are given in Table 3. The portion of bacterial N on total faecal N in the pollen group was about 9 and 6 percentage points lower than in the control and the wheat bran groups, whereas the portion of the endogenous N was about 6 and 5 percentage points higher, respectively. However, differences between the means were not significant. Similarly, daily excretion of bacterial N in the pollen group was numerically higher than in the control and the wheat bran group. The daily excretion of endogenous and undigested dietary N in the pollen group (0.97 and 0.39 g/d, respectively) was higher ($p < 0.05$) than in the control group (0.42 and 0.19 g/d, respectively), whereas the wheat bran group showed intermediate values of 0.58 and 0.25 g/d, respectively.

Given that the mean daily N-intake of 34 g and the mean daily urinary N-excretion of 16.1 g showed no variation between groups, the increased ($p < 0.05$) daily faecal N-excretion of animals of the pollen group resulted in a slightly lower N-retention compared to the control and the wheat bran group.

The dietary treatment had only minor influences on bacterial counts of the faeces (Table 3). However, for all bacterial species, lowest counts were observed in faeces of the control group, and highest values in faeces of the Pine pollen group. Moreover, compared to the pollen group, the daily faecal excretion of *Enterococci*, *E. Coli*, *lactic acid* bacteria, *Clostridium perfringens* and *Bifidobacterium spp.* were decreased ($p < 0.05$) in the control and the wheat bran group, respectively.

The concentration of different faecal lipids was highly dependent on the type of diet. The faecal triacylglycerol concentration in the pollen group was 72 and 120 percentage points increased ($p < 0.05$) compared to the control and the wheat bran group, respectively, whereas the tocopherol concentration was decreased ($p < 0.05$; Table 4). The faecal concentration of bile acids in animals of the control group was increased ($p < 0.05$) by 2.04 and 2.68 mg/g compared to the pollen and the wheat bran group, respectively, whereas faecal cholesterol concentration showed no differences be-

Table 2: Food intake, amount of daily excreted faeces and faecal crude nutrient composition[1]
Tabelle 2: Futteraufnahme, tägliche faecale Exkretion und Rohnährstoffzusammensetzung der Faeces[1]

	Treatment		
	Control	Pollen	Wheat bran
Food intake (g DM/d)	1111 ± 78	1101 ± 84	1168 ± 75
Amount of excreted faeces (g/d)			
Fresh matter	98 ± 24 [b]	194 ± 59 [a]	109 ± 22 [b]
Dry matter	59 ± 17 [b]	97 ± 25 [a]	64 ± 7 [b]
Crude nutrient content of faeces			
Dry matter (%)	60.0 ± 4.4 [a]	50.6 ± 4.8 [b]	60.1 ± 7.5 [a]
Crude ash (% of DM)	32.4 ± 2.7 [a]	23.4 ± 2.7 [b]	24.6 ± 2.2 [b]
Crude protein (% of DM)	18.0 ± 2.5	19.6 ± 2.8	20.5 ± 3.9
Crude lipids (% of DM)	11.3 ± 2.4	10.8 ± 0.9	10.1 ± 1.0
Crude fibre (% of DM)	22.7 ± 2.1 [b]	26.2 ± 2.2 [a]	21.6 ± 2.9 [b]
N-free extracts (% of DM)	15.6 ± 2.2 [c]	20.0 ± 1.1 [b]	23.1 ± 1.3 [a]

[1] Results are means ± SD. Means within a row not sharing the same superscript letters differ significantly by Student-Newman-Keuls-test ($p < 0.05$). Abbreviations: DM = Dry Matter, d = day

Table 3: Faecal nitrogen composition, nitrogen balance and faecal germ contents[1]
Tabelle 3: N-Fraktionen in den Faeces, N-Bilanz und Keimzahlen in den Faeces[1]

	Treatment		
	Control	Pollen	Wheat bran
Fractions of faecal nitrogen (% of total N)			
Bacterial N	63.3 ± 7.8	54.5 ± 7.0	60.3 ± 5.5
Endogenous N	26.4 ± 10.9	32.6 ± 7.0	27.5 ± 5.1
Undigested dietary N	10.2 ± 4.8	12.9 ± 3.2	12.2 ± 3.8
Excretion of faecal nitrogen (g/d)			
Bacterial N	1.09 ± 0.44	1.64 ± 0.48	1.27 ± 0.33
Endogenous N	0.42 ± 0.12 ^{b}	0.97 ± 0.31 ^{a}	0.58 ± 0.19 ^{ab}
Undigested dietary N	0.19 ± 0.15 ^{b}	0.39 ± 0.15 ^{a}	0.25 ± 0.05 ^{ab}
Nitrogen balance (g/d)			
Intake	33.2 ± 2.3	33.3 ± 2.5	34.5 ± 2.2
Faecal excretion	1.7 ± 0.6 ^{b}	3.0 ± 0.7 ^{a}	2.1 ± 0.4 ^{b}
Urinary excretion	15.7 ± 1.9	16.3 ± 1.7	16.3 ± 1.9
Retention	15.8 ± 1.4	14.0 ± 1.8	16.1 ± 1.9
Faecal Germ counts (Log_{10} CFU/g DM)			
Enterococci	9.83 ± 1.06	11.64 ± 1.49	11.11 ± 1.37
E. coli	12.24 ± 1.63	14.44 ± 1.29	12.99 ± 1.14
Clostridium perfringens	8.59 ± 0.92	10.36 ± 2.60	8.48 ± 1.05
Lactic acid bacteria	11.03 ± 1.78	12.65 ± 2.62	12.83 ± 1.29
Bifidobacterium spp.	11.38 ± 1.61	13.34 ± 1.74	12.76 ± 1.32
Excretion of faecal germs (Log_{10} CFU/d)			
Enterococci	571 ± 133 ^{b}	1120 ± 285 ^{a}	717 ± 154 ^{b}
E. coli	710 ± 160 ^{b}	1392 ± 343 ^{a}	831 ± 124 ^{b}
Clostridium perfringens	531 ± 124 ^{b}	911 ± 92 ^{a}	537 ± 87 ^{b}
Lactic acid bacteria	638 ± 137 ^{b}	1202 ± 321 ^{a}	826 ± 160 ^{b}
Bifidobacterium spp.	663 ± 169 ^{b}	1301 ± 403 ^{a}	823 ± 166 ^{b}

[1] Results are means ± SD. Means within a row not sharing the same superscript letters differ significantly by Student-Newman-Keuls-test ($p < 0.05$). Abbreviations: DM = Dry Matter, d = day

tween the treatments. As a result of varying amounts of faeces and faecal concentration of triglycerides and cholesterol, the daily excretion of these lipid fractions was similar in the control and the wheat bran group, but increased ($p < 0.05$) in animals of the pollen group. Daily faecal excretion of bile acids in animals of the pollen group of 415 mg was higher ($p < 0.05$) compared to animals of the wheat bran group (246 mg/d), whereas animals of the control group had an intermediate daily excretion of 372 mg. Daily tocopherol excretion was highest in animals of the pollen group, but differences between treatments were not significant.

The concentration of the sum of fatty acids in the faeces of pollen group of 15.78 mg/g was higher ($p < 0.05$) compared to the control and the wheat bran group, respectively (9.07 and 8.81 mg/g). This higher faecal total fatty acid concentration in the pollen group was mainly a result from increased ($p < 0.05$) faecal concentrations of 16:0, 18:1*n*-9 and 18:2*n*-6 fatty acids, respectively.

The daily excretion of 16:0, 18:0, 18:1*n*-9, 18:2*n*-6, 20:1*n*-9 and 20:5*n*-3 fatty acids in animals of the pollen group was increased ($p < 0.05$) compared to the other treatments, whereas daily excretion of other fatty acids as 12:0, 18:1*n*-7, 18:3*n*-3 or 24:0 was only marginally higher. In total, fatty acid excretion in the control and the wheat bran group was about 75 and 72 percentage points lower ($p < 0.05$) than in the pollen group (1506 mg/d).

As a result of the comparable intake level of different dietary crude nutrients and the high variability in faecal excretion between the treatments, apparent digestibility of DM, CP, CL and NfE was decreased ($p < 0.05$) in animals of the pollen group (Table 5). The same tendency was observed for digestibilities of CA and CF, respectively, but, however, differences were not significant. Consequently, the dietary metabolizable energy content calculated from the dietary digestible nutrient concentration, was reduced ($p < 0.05$) in the pollen diet compared to the other diets.

Table 4*:* Faecal lipid composition and excretion of lipids with faeces[1]
Tabelle 4: Lipidfraktionen in den Faeces und tägliche faecale Lipidexkretion[1]

	Treatment		
	Control	Pollen	Wheat bran
Content of lipids in faeces (DM)			
Triacylglycerols (mg/g)	4.32 ± 1.61 ^{b}	7.43 ± 1.21 ^{a}	3.37 ± 1.46 ^{b}
Cholesterol (mg/g)	7.13 ± 1.42	7.59 ± 0.99	6.70 ± 1.66
Bile acids (mg/g)	6.49 ± 2.70 ^{a}	4.45 ± 1.07 ^{b}	3.81 ± 1.37 ^{b}
Tocopherols (µg/g)	607 ± 83 ^{a}	471 ± 67 ^{b}	555 ± 44 ^{a}
Faecal excretion of lipids			
Triacylglycerols (mg/d)	242 ± 70 ^{b}	702 ± 125 ^{a}	208 ± 64 ^{b}
Cholesterol (mg/d)	408 ± 86 ^{b}	735 ± 201 ^{a}	421 ± 77 ^{b}
Bile acids (mg/d)	372 ± 155 ^{ab}	415 ± 69 ^{a}	246 ± 102 ^{b}
Tocopherols (mg/d)	35.4 ± 8.8	46.3 ± 16.5	35.4 ± 4.6
Fatty acid content in faeces (mg/g DM)			
12:0	0.02 ± 0.02	0.03 ± 0.03	0.02 ± 0.02
14:0	0.13 ± 0.04 ^{b}	0.18 ± 0.04 ^{ab}	0.21 ± 0.07 ^{a}
14:1*n*-5	0.15 ± 0.05	0.19 ± 0.07	0.15 ± 0.05
16:0	2.60 ± 0.67 ^{b}	4.63 ± 0.64 ^{a}	2.56 ± 0.52 ^{b}
18:0	4.21 ± 1.86	4.74 ± 1.11	3.65 ± 0.63
18:1*n*-9	0.50 ± 0.14 ^{b}	3.23 ± 0.39 ^{a}	0.70 ± 0.35 ^{b}
18:1*n*-7	0.10 ± 0.05	0.13 ± 0.12	0.13 ± 0.07
18:2*n*-6	0.22 ± 0.06 ^{c}	1.52 ± 0.13 ^{b}	0.39 ± 0.11 ^{a}
18:*3n*-3	0.06 ± 0.05	0.09 ± 0.07	0.08 ± 0.06
20:1*n*-9	0.07 ± 0.02 ^{b}	0.12 ± 0.03 ^{a}	0.06 ± 0.02 ^{b}
20:5*n*-3	0.48 ± 0.06	0.47 ± 0.09	0.41 ± 0.08
24:0	0.51 ± 0.07 ^{a}	0.43 ± 0.07 ^{b}	0.41 ± 0.05 ^{b}
22:5*n*-3	0.03 ± 0.02	0.03 ± 0.02	0.04 ± 0.01
Total	9.07 ± 2.62 ^{b}	15.78 ± 1.96 ^{a}	8.81 ± 0.59 ^{b}
Faecal excretion of fatty acids (mg/d)			
12:0	1 ± 1	3 ± 4	1 ± 1
14:0	8 ± 3 ^{b}	18 ± 6 ^{a}	13 ± 4 ^{ab}
14:1*n*-5	9 ± 4	19 ± 10	10 ± 3
16:0	155 ± 61 ^{b}	444 ± 110 ^{a}	161 ± 22 ^{b}
18:0	249 ± 121 ^{b}	452 ± 128 ^{a}	233 ± 43 ^{b}
18:1*n*-9	28 ± 5 ^{b}	307 ± 55 ^{a}	43 ± 18 ^{b}
18:1*n*-7	6 ± 4	11 ± 8	9 ± 5
18:2*n*-6	13 ± 3 ^{b}	145 ± 27 ^{a}	25 ± 9 ^{b}
18:*3n*-3	4 ± 5	9 ± 6	5 ± 3
20:1*n*-9	4 ± 1 ^{b}	11 ± 3 ^{a}	4 ± 2 ^{b}
20:5*n*-3	28 ± 6 ^{b}	44 ± 8 ^{a}	27 ± 8 ^{b}
24:0	30 ± 10	41 ± 11	26 ± 6
22:5*n*-3	2 ± 1	3 ± 2	3 ± 1
Total	535 ± 205 ^{b}	1506 ± 323 ^{a}	560 ± 33 ^{b}

[1] Results are means ± SD. Means within a row not sharing the same superscript letters differ significantly by Student-Newman-Keuls-test ($p < 0.05$). Abbreviations: DM = Dry Matter, d = day

4 Discussion

Dietary fibres represent a group of nutrients from plant materials which are thought to be essential components in human as well as in animal nutrition. Besides lignin, they consist of a number of carbohydrates as celluloses, hemicelluloses, pectines and oligosaccharides, which are not degraded by enzymes of the human gastrointestinal tract. Moreover, resistant starch should be classified as a dietary fibre (AMERICAN ASSOCIATION OF CEREAL CHEMISTS, 2001). Given that dietary fibres are partly degraded to volatile fatty acids by the microflora of the lower gut, they play, besides

Table 5: Crude nutrient digestibilities and dietary metabolizable energy[1]
Tabelle 5: Rohnährstoffverdaulichkeit und umsetzbare Energie der Rationen[1]

	Treatment		
	Control	Pollen	Wheat bran
Apparent digestibility of nutrients (%)			
Dry matter	94.7 ± 1.4 [a]	91.2 ± 2.1 [b]	94.5 ± 0.9 [a]
Crude ash	55.9 ± 12.3	44.5 ± 19.4	62.3 ± 8.8
Crude protein	94.9 ± 1.5 [a]	91.0 ± 2.0 [b]	93.9 ± 1.3 [a]
Crude lipids	85.2 ± 2.5 [a]	76.4 ± 5.5 [b]	86.4 ± 1.4 [a]
Crude fibre	55.5 15.2	40.5 11.7	59.3 11.0
N-free extracts	98.8 0.4 [a]	97.5 0.6 [b]	98.2 0.2 [a]
Metabolizable energy (MJ ME/kg DM)[2]	17.3 ± 0.2 [a]	16.7 ± 0.3 [b]	17.3 ± 0.1 [a]

[1] Results are means ± SD. Means within a row not sharing the same superscript letters differ significantly by Student-Newman-Keuls-test ($p < 0.05$)
[2] Calculated from digestible nutrients
Abbreviations: DM = Dry Matter, ME = metabolizable energy

other essential functions, a role in regulation of the pH of the chyme, provide nutrients for the intestinal mucosa and, in the case of absorption of the volatile fatty acids, they serve as an additional energy source.

In Germany, the recommended dietary fibre intake is 12.5 and 10.0 g/1000 kcal for women and men, respectively (DACH, 2000). Comparable to the US, where the dietary fibre intake was only 6.5 and 5.5 g/1000 kcal in women and men, respectively (LANZA et al., 1987), the daily intake of dietary fibres is far below the current recommendations in Germany (ADOLF et al., 1994) and other developed countries (SPILLER, 2001). In order to reflect this lack of dietary fibre in so called "western style diets" with a high nutrient and energy density, the present experiment used a semisynthetic control diet based mainly on corn starch, sugar, soybean protein isolate and small amounts of purified cellulose, which provided a low dietary fibre concentration of approximately 6.6 g/1000 kcal. On the other hand, the supplementation level of 3.5 % Pine pollen (and wheat bran) was chosen to reflect the upper range of typical inclusion levels of Pine pollen in a Chinese diet. Thus, we evaluated the effects of addition of the different fibre sources pollen and wheat bran at similar levels to a low fibre control diet (Table 1). Consequently, dietary fibre concentrations of the pollen and the wheat bran diet of approximately 10 g/1000 kcal, respectively, were higher compared to the control diet, but remained, however, below the above mentioned recommendations for male individuals.

A main finding of the present study was the high ($p < 0.05$) increase in faeces weight, both on a fresh (about 100%) and a DM (about 65 %) basis, due to inclusion of pollen in the diet. This bulking effect is in accordance to the use of Pine pollen in the traditional Chinese medicine for the cure of chronic constipation in men (ZHANG, 1993) and an increase in stool weight may affect health in several ways. It has been shown, that the effect of wheat bran on stool weight depends on the particle size of the bran (BRODRIBB and GROVES, 1978) and therefore the small diameter of the pollen (Figure 1) may attribute to the observed bulking effect. However, given that it is known that stool weight increases as intake of fibre increases (CUMMINGS, 1993; HAACK et al., 1998; SOUTHGATE and DOURNIN, 1970), it remains unclear whether the observed increase in faecal output is an effect directly attributed to the properties of Pine pollen rather than to the elevated dietary fibre concentration. Nevertheless, previous studies (ZHAO et al., 1994, 1996, 2000; ZHAO and BAO 2001) have shown that Masson Pine pollen contained about 29 % lignin, 14 % celluloses, 13 % hemicelluloses and only 15 % of soluble carbohydrates. Given that there is no increase in stool weight, if the fibre is fully and rapidly fer-

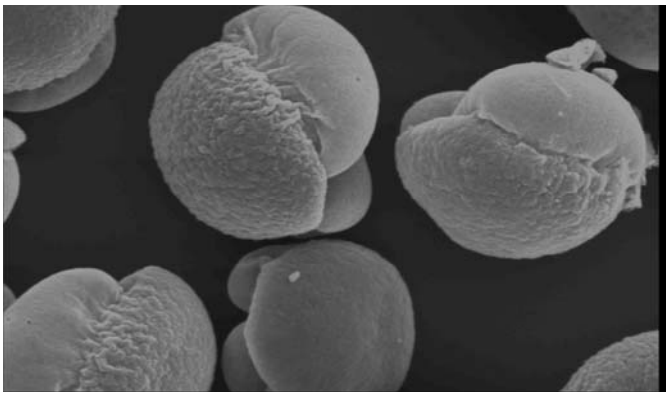

Figure 1: Electronic microscopy of native Masson Pine pollen (magnification, 1:800)
Abbildung 1: Elektronenmikroskopische Aufnahme von nativen Masson Pinienpollen (Vergrößerung, 1:800)

mented in the large bowel (CUMMINGS, 1993), probably a high fraction of insoluble fibre in the Pine pollen may be responsible for the observed effects.

Despite the observed effect on daily excretion of faeces, DM content of faeces was highly ($p < 0.05$) reduced due to inclusion of pollen in the diet (Table 2) and this low DM content resulted in a soft and shapeable appearance of faeces compared to the other treatments. In men, the DM content of stool is about 25 to 30 %, and the relative composition seems not to vary with an increase of stool weight due to an enhanced dietary fibre intake (TREPEL, 2004). Even if the exact composition of the Pine pollen is not known, it seems to have some properties which may increase the water content of faeces.

In the present study, neither addition of Pine pollen nor addition of wheat bran to the diets did significantly shift the faecal microbial pattern. Based on former studies in rats (ZHAO et al., 1996), which demonstrated broken Pine pollen to suppress the facultative pathogen germs *Proteus mirabilis* and *Escherichia Coli*, respectively, one of our assumptions was a kind of prebiotic action of Pine pollen. Nevertheless, as shown in Table 3 the daily faecal excretion of all analysed kinds of bacteria were increased ($p < 0.05$) in the pollen group compared to the control and the wheat bran group, respectively. Moreover, faecal output of crude protein was increased, whereas the digestibility of nitrogen was decreased, but since faecal protein is mainly of bacterial origin, this fact reflects the extent of hindgut fermentation rather then a depressive effect on intestinal availability of amino acids (KREUZER et al., 1991). In summary, those results indicate the potential of pollen to stimulate bacterial growth in the gut, but however, from the present results in pigs this stimulation appears not to be directed selectively to *bifidobacteria* and *lactobacilli*, a defining effect of prebiotics (CUMMINGS and MACFARLANE, 2002). As shown in Figure 1 the whole particles of native Masson Pine pollen are about 40 µm in diameter and consist of a main part covering two *sacci*, which serve as "airbags" for the anemogamic pines. Thus, as reflected by the small diameter, the Masson Pine pollen represents a very fine material which provides, in turn, a huge surface what may ease attraction of gut bacteria. On the other hand, as shown in Figure 2, there seems to be a large part of pollen material (mainly the "airbags") which is neither digested by the host enzymes nor degraded by the microflora, and this fact is also reflected by the lower DM and CF digestibility (Table 5) of the pollen diet.

Faecal concentration of bile acids and tocopherols in animals of the pollen group was decreased compared to animals of the control and the wheat bran group, but daily excretion of triacylglycerols, cholesterol and bile acids was increased ($p < 0.05$) in animals of the pollen group compared to animals of the wheat bran group, respectively (Table 4). Those figures, which may correspond to the serum and liver cholesterol lowering effect of different fibre sources (ANDERSON et al., 1994), are related to the soluble fraction of dietary fibre (SCHAARMANN et al., 1999), rather than to the insoluble fraction. In rats it was shown that feeding psyllium-containing diets resulted in lowering of plasma cholesterol concentration, but increased bile acid excretion (TERPSTRA et al., 2000), what may be a result of an increased viscosity of intestinal contents. We did not investigate serum lipid fractions in the present investigation, but

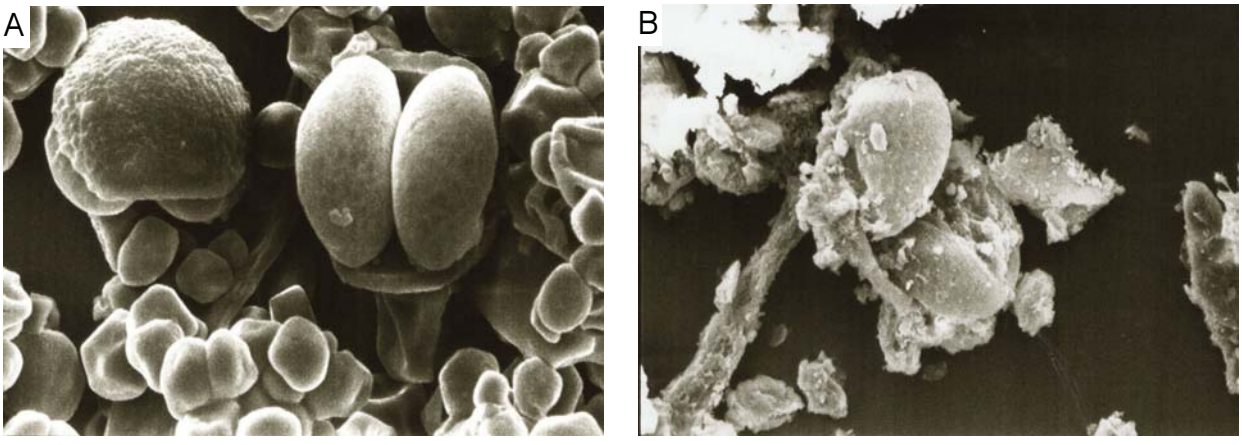

Figure 2: Electronic microscopy of Masson Pine pollen (A) in the diet, (B) in faeces (magnification, 1:800)
Abbildung 2: Elektronenmikrokopische Aufnahme von Masson Pinienpollen (A) in der Ration, (B) in den Faeces (Vergrößerung, 1:800)

the altered faecal lipid pattern and excretion suggests that the soluble fibre fraction of Masson Pine pollen may be comparable to other fibre sources, which alter bile acid excretion and bile acid metabolism in humans (EVERSON et al., 1992; MIETTINEN and TARPILLA, 1989) or alter serum cholesterol in rats and humans (ANDERSON et al., 1991; JENNINGS et al., 1988).

In conclusion, Masson Pine pollen represents a dietary fibre complex used in traditional Chinese medicine, which is especially rich in indigestible carbohydrates. A 3.5 % addition of Pine pollen to a high nutrient density diet increases stool weight and water content of stools what may correspond to the benefits in the cure of chronic constipation in men. Furthermore, the pollen additive seems to stimulate development of gut microflora, but in contrary to former observations in rats this effect was not specifically directed to bacterial species thought to be beneficial for eubiosis in the hindgut of men and animals. Given that the inclusion of pollen in the diet seems to alter the nitrogen and lipid metabolism, it may be concluded that the soluble fraction of the pollen fibre has similarities to different fibre fractions thought to have positive influences on bile acid and cholesterol metabolism in men. Therefore, from the present results it is concluded, that Masson Pine pollen is a suitable dietary additive to elevate dietary supply of both, indigestible and soluble fractions of dietary fibre in men. However, further studies are required to investigate more detailed effects of Masson Pine pollen on metabolism in men and animals.

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Abbreviations used

BW, Body weight
CA, Crude Ash
CF, Crude Fibre
CFU, colony forming units
CL, Crude Lipids
CP, Crude Protein
DM, Dry Matter
ME, Metabolizable Energy
N, Nitrogen
NfE, Nitrogen free Extract
SD, standard deviation

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