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## Originalien

- Dr. W. Nicklas: **Mikrobiologische Standardisierung bei Versuchstieren** 201  
☐ Laboratory animals ☐ microbiological standardisation  
☐ microbiological quality ☐ management, ☐ health monitoring
- M. Awad-Masalmeh, I. Zimernik, W. Baumgartner, F. Hinterdorfer und J. Flatscher: **Nachweis von *Mycobacterium avium subspecies paratuberculosis* (*M. p.*) mittels Polymerase-Kettenreaktion (PCR) und Kulturversuch (KV) bei Tieren in Österreich** 211  
☐ *M. avium* subsp. *Paratuberculosis* ☐ ruminants ☐ PCR ☐ culture
- P. Winter, A. Höflechner, W. Baumgartner: **Zum Resistenzverhalten von ovinen Mastitisserregern** 216  
☐ mastitis ☐ minimal inhibitory concentration ☐ ovine ☐ resistance
- I. Yeruham and U. Orgad: **Case Report: Primary Fibrosarcoma of the Liver in a Cow** 223  
☐ Fibrosarcoma ☐ liver ☐ diagnostics ☐ cow
- R. Nemcová, A. Bomba, S. Gancarčíková, R. Herich, P. Guba: **Der Einfluß der Applikation von *Lactobacillus paracasei* und Fruktooligosacchariden auf die Kotmikroflora der Absatzferkel** 225  
☐ *Lactobacillus paracasei* ☐ fructooligosaccharides ☐ broiler ☐ faecal microflora  
☐ weanling piglets
- E. Neubert, G. Gründel: **Altersabhängigkeit der Dichte  $\alpha_1$ -adrenerger Rezeptoren an Leberzellmembranen beim Haushuhn** 229  
☐  $\alpha_1$ -adrenergic receptors ☐ hepatocytes ☐ chickens ☐ age dependence
- M. Röhrs und P. Ebinger: **Verwildert ist nicht gleich wild: Die Hirngewichte verwilderter Haussäugetiere** 234  
☐  $\alpha_1$ -adrenergic receptors ☐ hepatocytes ☐ chickens ☐ age dependence
- M. Hertel, U. Kersten, R. Mischke und I. Nolte: **Das Langzeit-EKG beim Hund: Vergleich zwischen computergesteuerter und visuell ausgewerteter Arrhythmieanalyse** 239  
☐ ECG ☐ holter monitoring ☐ arrhythmia ☐ dog
- A. Klein, A. Adamik und R. Mischke: **Lagerungsbedingte Veränderungen im Thrombozytenkonzentrat des Hundes. Teil I: Zahl und In-vitro-Funktionsfähigkeit der Thrombozyten** 243  
☐ platelet concentrate ☐ dog ☐ storage ☐ platelet count ☐ aggregation  
☐ resonance-thrombograph
- P. Reinhold, A. Langenberg, G. Becher, M. Rothe: **Das Atemkondensat – ein nichtinvasiv zu gewinnendes Medium zum Nachweis von Entzündungsmediatoren der Lunge** 254  
☐ exhaled condensate ☐ leukotriene B<sub>4</sub> (LTB<sub>4</sub>) ☐ pneumonia, diagnosis ☐ calf

## Klinische Pharmakologie und Diagnostik

## Rubriken

*b*

- ☐ Neue Bücher 260 ☐ Tagungen und Sitzungen 264  
☐ Tagungsberichte 265 ☐ Aus der Industrie 268  
☐ Kleine Mitteilungen 269 ☐ Von Universitäten und  
Hochschulen 277 ☐ Persönliches 279 ☐ Vorschau 280



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## Study of the effect of *Lactobacillus paracasei* and fructooligosaccharides on the faecal microflora in weanling piglets

Der Einfluß der Applikation von *Lactobacillus paracasei* und Fruktooligosacchariden auf die Kotmikroflora der Absatzferkel

R. Nemcová, A. Bomba, S. Gancarčíková, R. Herich, P. Guba

**Summary:** The influence of administration of *Lactobacillus paracasei* alone and mixture of *Lactobacillus paracasei* and fructooligosaccharide on faecal bacteria counts in the weanling pigs was investigated. The administration of *Lactobacillus paracasei* alone significantly decreased *Clostridium* ( $p < 0.05$ ) and *Enterobacteriaceae* ( $p < 0.05$ ) counts as compared to the control. *Lactobacillus paracasei* administered in combination with fructooligosaccharide significantly increased *Lactobacillus* ( $p < 0.01$ – $p < 0.05$ ), *Bifidobacterium* ( $p < 0.05$ ), total anaerobes ( $p < 0.05$ ), and total aerobes ( $p < 0.05$ ) counts compared to control group as well as *Lactobacillus paracasei* group and significantly decreased *Clostridium* ( $p < 0.05$ ) and *Enterobacteriaceae* ( $p < 0.01$ ) counts compared to control group. The results obtained point out to a synergic effect of the combination of *Lactobacillus paracasei* and fructooligosaccharide on numbers of bacterial populations observed in the faeces of the weanling pigs.

**Key Words:** *Lactobacillus paracasei*, fructooligosaccharides, faecal microflora, weanling piglets

**Zusammenfassung:** In der vorliegenden Arbeit wurde der Einfluss der Applikation von *Lactobacillus paracasei* allein und der Mischung von *Lactobacillus paracasei* und Fruktooligosacchariden auf die Zahlen der Kotbakterien bei den Absatzferkeln untersucht. Die Inokulation von *Lactobacillus paracasei* allein erniedrigte die Zahlen von *Clostridium* ( $p < 0.05$ ) und *Enterobacteriaceae* ( $p < 0.05$ ) gegenüber der Kontrollgruppe signifikant. *Lactobacillus paracasei*, der in der Mischung mit Fruktooligosacchariden appliziert wurde, hat die Zahlen von *Lactobacillus* ( $p < 0.01$  bis  $p < 0.05$ ), *Bifidobacterium* ( $p < 0.05$ ), die Gesamtkeimzahl der aeroben Bakterien ( $p < 0.05$ ) und der anaeroben Bakterien ( $p < 0.05$ ) signifikant gegenüber der Kontrollgruppe und der *Lactobacillus paracasei* Gruppe erhöht. Gleichzeitig hat die Mischung von *Lactobacillus paracasei* und Fruktooligosacchariden die Zahlen von *Clostridium* ( $p < 0.05$ ) und *Enterobacteriaceae* ( $p < 0.01$ ) gegenüber der Kontrollgruppe erniedrigt. Die Ergebnisse zeigen den synergistischen Effekt der Kombination von *Lactobacillus paracasei* und Fruktooligosacchariden auf die Zahlen der Bakterienpopulationen in den Kotproben von Absatzferkeln.

**Schlüsselworte:** *Lactobacillus paracasei*, Fruktooligosaccharide, Kotmikroflora, Absatzferkel

### Introduction

Promoting animal health, improving growth rate and feed efficiency through modification of microbial fermentation in the gastrointestinal tract of monogastric animals is a subject of considerable interest. The diverse collection of microorganisms colonising the healthy gastrointestinal tract of pigs plays an essential role both for the well-being of the animal and for animal nutrition and performance as well as for the quality of animal products. A number of naturally-occurring and artificial factors have been shown to affect the composition and activity of the microbiota in the gastrointestinal tract of pigs, these include: diet composition, growth promoting antibiotics, copper, use of probiotics, specific carbohydrates, organic acids, and fermented feed (Jensen, 1998).

Probiotics, including yeast, lactobacilli, bacilli, streptococci, have been reported to improve intestinal microbial balance of neonatal and weaned pigs (Muralidhara *et al.*, 1977, Gedek, 1986, Mathew *et al.*, 1998). They could be defined as selected and concentrated viable counts of beneficial bacteria and yeast,

that are administered orally, either alone or in feed, with the intent of establishing a favourable intestinal microflora, in order to prevent digestive disorders and/or to increase performance (Fuller, 1989). Specific carbohydrates, especially fructooligosaccharides (FOS), are naturally occurring oligosaccharides, mainly of plant origin. They have been shown to be resistant to endogenous glycolytic enzymes of the host and to pass unaltered to the colon (Oku *et al.*, 1984). FOS can significantly modulate the colonic microbiota by increasing the specific bacteria count and thus changing the composition of the microbiota.

The concept of "synbiotics" (mixture of probiotic and oligosaccharide) has recently been proposed to characterise health-enhancing foods and supplements used as functional food ingredients in humans (Gibson and Roberfroid, 1995, Kontula *et al.*, 1998). When combining both a probiotic and oligosaccharide, the expected benefits are an improved survival during the passage of the probiotic bacteria through the upper intestinal tract and a more efficient implantation in the colonic microbiota together with a stimulating effect of the



oligosaccharide on the growth and/or the activities of both the exogenous (probiotic) and endogenous bacteria (Roberfroid, 1998). Biological preparation containing *Enterococcus faecium* M-74 and mannan-oligosaccharides can have beneficial effect on pig growth and feed conversion (Kumprecht and Zobač, 1998). Mixed probiotics containing *Lactobacillus fermentum*, *Enterococcus faecium*, lactulose and lactitol, improved daily gains and decreased mortality of piglets (Noussainen and Setälä, 1993).

The aim of the present study was to observe the effect of the administration of *Lactobacillus paracasei* and mixture of *Lactobacillus paracasei* and fructooligosaccharide on bacteria counts in the faeces of the weanling pigs under field conditions.

### Materials and Methods

The study with piglets, crossbreeds Landrace, Yorkshire and Piétrain breeds weaned at the age of 36 days, was conducted at large-scale pig farm. The trial involved piglets of average live weight 7 kg. Piglets were kept in 9 cages, ten animals per cage. The cages were placed in one stall. Throughout the study, the animals were fed feed mixtures for early weaning of piglets (ČOS, Pol'nonákup Spiš, Spišské Vlachy, Slovak Republic). 3 experimental groups were used in trial:  
group 1 (3 cages) – control without any additives  
group 2 (3 cages) –  $1 \times 10^9$  of *Lactobacillus paracasei* per 1 g  
group 3 (3 cages) –  $1 \times 10^9$  of *Lactobacillus paracasei* per 1 g and FOS (Raftilose P95).

Raftilose P95 was manufactured by Raffinerie Tirlemontoise, Tienen, Belgium. *Lactobacillus paracasei* was isolated from the intestine of one week-old piglets and characterized at our laboratory (Nemcová *et al.*, 1997). Additives were administered per os immediately on day 1 of weaning throughout 10 days. The experimental animals received FOS daily at a dose of 3 g and *Lactobacillus paracasei* daily at a dose of 2 g for one weanling pig.

20 days after the beginning of the study, fresh samples of faeces were taken from 7 animals from each group. 1g of faeces was placed in sterile polyethylene Stomacher Lab Blender bag (Seward Medical Limited, London, UK) with 9 ml of sterile anaerobic diluent (0.4 g NaHCO<sub>3</sub>, 0.05 g L-cysteine HCl, 1 ml resazurin /0.1%/, 7.5 ml mineral solution I/0.6 % K<sub>2</sub>HPO<sub>4</sub>/, 7.5 ml mineral solution II/1.2 % NaCl, 1.2 % (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.6 % KH<sub>2</sub>PO<sub>4</sub>, 0.12 % CaCl<sub>2</sub>, 0.25 % MgSO<sub>4</sub>/ and 84 ml distilled water, pH 6.8) and stomached (Stomacher Lab Blender 80, Seward Medical Limited, London, UK) for 2 min under a CO<sub>2</sub> atmosphere. Series of 10-fold dilutions ( $10^{-2}$  to  $10^{-8}$ ) were made in the same diluents. From appropriate dilutions, 0.1 ml aliquots were spread onto two nonselective agar plates: trypticase soy blood agar with 10 % sheep blood (BBL, Microbiology systems, Cockeysville, USA) for aerobes and Schaedler agar with 1 % vitamin K<sub>1</sub> – hemin solution (BBL) for anaerobes. Aliquots (0.1 ml) were also spread on 6 selective agar media: Beerens medium (Beerens, 1990) for *Bifidobacterium*, Rogosa agar (Imuna, Šarišské Michal'any, Slovakia) for *Lactobacillus*, Enterococcosel agar (BBL) for *Enterococcus*, Clostrisel agar (BBL) for *Clostridium*, MacConkey agar (Imuna) for *Coliforms* and Endo agar (Imuna) for *Enterobacteriaceae*. For anaerobes, plates containing the media were kept in the anaerobic jars for 24 h before analysis. Incubation of the inoculated media for anaerobic bacteria was

carried out at 37 °C for 4 days under anaerobiosis (Gas Pak Plus, BBL). Plates for the enumeration of aerobic bacteria were incubated for 2 days at 37 °C. Colonies were counted and bacteria were Gram stained and visualized under the microscope for morphological characterization. Total anaerobic counts were corrected by evaluating aerotolerance of the different colony types. The viable counts are expressed as the log 10 of colony-forming units (CFU) g<sup>-1</sup> of faeces. The results are presented as mean values  $\pm$  SEM. Statistical significance was determined by the t-test.

### Results

Numbers of individual bacterial populations found in both experimental and control animals are presented in Table 1. In faeces of experimental animals receiving the mixture of *Lactobacillus paracasei* and FOS, significantly higher *Lactobacillus* ( $p < 0.01$ ), *Bifidobacterium* ( $p < 0.05$ ), total anaerobes ( $p < 0.05$ ), and total aerobes ( $p < 0.05$ ) counts have been found as compared to the control and significantly higher anaerobes ( $p < 0.05$ ), total aerobes ( $p < 0.05$ ), *Bifidobacterium* ( $p < 0.05$ ), and *Lactobacillus* ( $p < 0.05$ ) counts compared to *Lactobacillus paracasei* group. Compared to the control, significant decrease in *Clostridium* ( $p < 0.05$ ), *Enterobacteriaceae* ( $p < 0.01$ ) counts was observed as well as an insignificant decrease in *Coliform* counts by 1 log. *Enterococcus* counts were significantly reduced ( $p < 0.001$ ) compared to both control group and *Lactobacillus paracasei* receiving group. In faeces of experimental animals receiving *Lactobacillus paracasei*, significant

Table 1  
Composition of faecal microflora in weanling pigs receiving *Lactobacillus paracasei* and mixture of *Lactobacillus paracasei* and FOS

Organisms	Group 1	Group 2	Group 3
Total anaerobes	9.8 $\pm$ 0.2	9.8 $\pm$ 0.3	10.2 $\pm$ 0.2 a*, b*
Total aerobes	8.0 $\pm$ 0.5	8.3 $\pm$ 0.2	9.3 $\pm$ 0.7 a*, b*
Bifidobacterium	7.5 $\pm$ 0.3	7.1 $\pm$ 0.7	8.2 $\pm$ 0.3 a*, b*
Lactobacillus	9.9 $\pm$ 0.1	9.9 $\pm$ 0.3	10.3 $\pm$ 0.1 a**, b*
Enterococcus	9.3 $\pm$ 0.1	9.3 $\pm$ 0.3	8.2 $\pm$ 0.2 a***, b***
Clostridium	8.1 $\pm$ 0.1	7.4 $\pm$ 0.4 a*	7.7 $\pm$ 0.3 a*
Enterobacteriaceae	7.9 $\pm$ 0.4	6.5 $\pm$ 0.9 a*	5.9 $\pm$ 0.9 a**
Coliforms	6.8 $\pm$ 0.7	6.3 $\pm$ 0.7	5.8 $\pm$ 0.7

Values are mean  $\pm$  SEM of log bacteria counts per gram of wet faeces (n = 7)

group 1 – control

group 2 – *Lactobacillus paracasei*

group 3 – *Lactobacillus paracasei* and FOS

a Significantly different from control group

b Significantly different from *Lactobacillus paracasei* group

\*  $p < 0.05$

\*\*  $p < 0.01$

\*\*\*  $p < 0.001$



decrease in *Clostridium* ( $p < 0.05$ ) and *Enterobacteriaceae* ( $p < 0.05$ ) counts as compared to the control was recorded. *Coliform* counts were by 0.5 log lower compared to control. This difference, however, was insignificant similarly like with *Coliforms* in previous experimental group due to the great variance of values in individual groups. *Lactobacillus*, *Enterococcus* and total anaerobes counts were identical in both groups. An insignificant increase in total aerobes in favour of experimental group was recorded and vice versa, there was an insignificant decrease in *Bifidobacterium* as compared to the control group.

## Discussion

As indicated by the results of the trial, the administration of *Lactobacillus paracasei* alone decreased *Clostridium* and *Enterobacteriaceae* counts, whereas the administration of *Lactobacillus paracasei* in combination with FOS has been manifested by a significant alteration in the numbers of all bacteria observed in faeces. The administration of *Lactobacillus paracasei* alone did not influence the concentration of lactobacilli. In our previous studies with gnotobiotic piglets we observed that the above-mentioned strain of *Lactobacillus paracasei* adhered to gut mucosa of both jejunum and ileum and survived in the intestinal tract (Nemcová *et al.*, 1998). We can suppose that unchanged fecal lactobacilli counts that we have observed may be associated with the above-mentioned ability of this strain to be adhered to gut mucosa.

FOS are known for their ability to stimulate the growth of bifidobacteria and lactobacilli under *in vitro* and *in vivo* conditions which belong to beneficial intestinal bacteria (Hidaka *et al.*, 1986, Mitsuoka *et al.*, 1987, Ito *et al.*, 1990, Sghir *et al.*, 1998). The above-mentioned fact is confirmed also by our observations. In faeces of animals receiving the mixture of *Lactobacillus paracasei* and FOS significantly higher *Lactobacillus* and *Bifidobacterium* counts were recorded compared to other groups observed. Lactic and acetic acids prove to be the main fermentation products of both lactobacilli and bifidobacteria. Some authors, however, claim that after fermentation of FOS by faecal inocula, succinate, propionate and butyrate could be detected (Wang and Gibson, 1993). The elevation in the concentrations of the above-mentioned acids may indicate the direct fermentation of FOS by other bacteria, or indirect fermentation of end products produced by lactobacilli and bifidobacteria. In our study we have also recorded higher level of butyric acids in animals receiving *Lactobacillus paracasei* and FOS (the results will be presented later on). This suggests that butyrate-producing bacteria are also involved in FOS fermentation. An increased butyrate production may be associated with significantly higher numbers of total anaerobes and aerobes observed by ourselves. These bacteria should be identified. Butyrate is known to be the major substrate for energy metabolism in colonic mucosa and it stimulates epithelial cell growth (Roediger, 1982). Hartemink *et al.* (1997) report that most enterobacteria in the human intestine are able to ferment FOS to a some extent. Morisse *et al.* (1993) were able to show a significant increase in saprophytic *E. coli* counts in rabbits with an FOS-containing diet. FOS has been shown to enhance the growth of *Bacteroides*, *Clostridium* and *Eubacterium* (Yamada *et al.*, 1993, Jaskari *et al.*, 1998).

In conclusion, the results of this study point to a synergic effect of *Lactobacillus paracasei* and FOS combination on faecal microflora of weaned pigs. This effect was demonstrated

by increased total anaerobes, aerobes, lactobacilli, and bifidobacteria counts as well as by decreased clostridia, *Enterobacteriaceae* and *E. coli* counts. The combination of probiotics and non-digestible carbohydrates may be a way of stabilization and/or potentiation of the effect of probiotics. Such potentiated probiotics indicate a realistic way of using biological preparations in the prevention of gastrointestinal diseases in weaned pigs.

## References

- Beerens, H. (1990): An elective and selective isolation medium for *Bifidobacterium* spp. *Letters Appl. Microbiol.* **11**, 155–157.
- Fuller, R. (1989): Probiotics in man and animals. *J. Appl. Bact.* **66**, 365.
- Gedek, B. (1986): Probiotics in animal feeding – effects on performance and animal health. *Feed Mag.*, 21–24.
- Gibson, G. R., and Roberfroid, M. B. (1995): Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *J. Nutr.* **125**, 1401–1412.
- Hartemink, R., Van Laere, K. M. J., and Rombouts, F. M. (1997): Growth of enterobacteria on fructo-oligosaccharides. *J. Appl. Microbiol.* **83**, 367–374.
- Hidaka, H., Eida, T., Takizawa, T., Tokunaga, T., and Tashiro, Y. (1986): Effects of fructo-oligosaccharides on intestinal flora and human health. *Bifidobacteria Microflora* **5**, 37–50.
- Ito, M., Deguchi, Y., Miyamori, A., Matsumoto, K., Kikuchi, H., Matsumoto, K., Kobayashi, Y., Yajima, T., and Kan, T. (1990): Effects of consumption of galacto-oligosaccharides on the human faecal microflora, stool weight and abdominal sensation. *Microbiol. Ecol. Health Dis.* **3**, 285–292.
- Jaskari, J., Kontula, P., Siitonen, A., Mattila-Sandholm, T., and Poutanen, K. (1998): Oat beta-glucan and xylan hydrolysates as selective substrates for probiotic strains of *Bifidobacterium* and *Lactobacillus*. *Appl. Microbiol. Biotechnol.* **49**, 175–181.
- Jensen, B. B. (1998): The impact of feed additives on the microbial ecology of the gut in young pigs. *J. Anim. Feed Sci.* **7**, 45–64.
- Kontula, P., Jaskari, J., Nollet, L., De Smet, I., von Wright, A., Poutanen, K., and Mattila-Sandholm, T. (1998): The colonization of a simulator of the human intestinal microbial ecosystem by a probiotic strain fed on a fermented oat bran product: effects on the gastrointestinal microbiota. *Appl. Microbiol. Biotechnol.* **50**, 246–252.
- Kumprecht, I., and Zobač, P. (1998): Study of the effect of a combined preparation containing *Enterococcus faecium* M-74 and mannan-oligosaccharides in diets for weanling piglets. *Czech J. Anim. Sci.* **43**, 477–481.
- Mathew, A. G., Chattin, S. E., Robbins, C. M., and Golden, D. A. (1998): Effects of a direct-fed yeast culture on enteric microbial populations, fermentation acids, and performance of weanling pigs. *J. Anim. Sci.* **76**, 2138–2145.
- Mitsuoka, T., Hidaka, H., and Eida, T. (1987): Effect of fructooligosaccharides on intestinal microflora. *Die Nahrung* **31**, 427–436.
- Morisse, J. P., Maurice, R., Boilletot, E., and Cotte, J. P. (1993): Assessment of the activity of a fructo-oligosaccharide on different caecal parameters in rabbits experimentally infected with *E. coli* 0.103. *Ann. Zootechnol.* **42**, 81–87.
- Muralidhara, K. S., Shedgeby, G. G., Elliker, P. R., England, D. C., and Sandine, W. E. (1977): Effect of feeding lactobacilli on the coliform and *Lactobacillus* flora of intestinal tissue and faeces from piglets. *J. Food Protection* **40**, 228.
- Nemcová, R., Lauková, A., Gancarčíková, S., and Kaštel', R. (1997): *In vitro* studies of porcine lactobacilli for possible probiotic use. *Berl. Munch. Tierärztl. Wschr.* **110**, 413–417.
- Nemcová, R., Bomba, A., Herich, R., and Gancarčíková, S. (1998): Colonization capability of orally administered *Lactobacillus* strains in the gut of gnotobiotic piglets. *Dtsch. Tierärztl. Wschr.* **105**, 199–200.
- Nousiainen, J., and Setälä, J. (1993): Lactic acid bacteria as animal probiotics. In: Salminen, S., von Wright, A. (eds.): *Lactic Acid Bacteria*. New York, USA, Marcel Dekker, 315–356.
- Oku, T., Tokunaga, T., and Hosoya, N. (1984): Nondigestibility of a new sweetener "Neosugar" in the rat. *J. Nutr.* **114**, 1574–1581.
- Roberfroid, M. B. (1998): Prebiotics and synbiotics: concepts and nutritional properties. *Brit. J. Nutr.* **80**, Suppl. 2, S197–S202.



- Roediger, W. E. W. (1982): Utilization of nutrients by isolated epithelial cells of rat colon. *Gastroenterology* **83**, 424–429.
- Sghir, A., Chow, J. M., and Mackie, R. I. (1998): Continuous culture selection of bifidobacteria and lactobacilli from human faecal samples using fructooligosaccharide as selective substrate. *J. Appl. Microbiol.* **85**, 769–777.
- Wang, X., and Gibson, G. R. (1993): Effects of the in vitro fermentation of oligofructose and inulin by bacteria growing in the human large intestine. *J. Appl. Bacteriol.* **75**, 373–389.
- Yamada, H., Itoh, K., Morichita, Y., and Taniguchi, H. (1993): Advances in cereal chemistry and technology in Japan. Structure and properties of oligomers from wheat bran. *Cereal Foods World* **38**, 492–499.

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