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Application of inulin-type fructans in animal feed and pet food

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The inulin-type fructans are non-digestible oligosaccharides that are fermented in the gastrointestinal tract of farm animals and pets. This review focuses on the various effects of inulin-type fructans in pigs, poultry, calves and companion animals. Effects of the inulin-type fructans on gut microflora, digestion and availability of nutrients, gut morphology, fermentation characteristics and animal performance are discussed. Inulin-type fructans can support animal performance and health by affecting nutrient digestion, gut microflora and gut morphology, although results vary depending on composition of the basal diet, inclusion level, type of fructan, adaptation period and experimental hygienic conditions.

Inulin-type fructans: Feed and pet food: Performance and health

For several decades, antibiotics and chemotherapeutics in prophylactic doses have been used in animal feed to improve animal welfare and to obtain economic benefits in terms of improved animal performance and reduced medication costs. However, there are increasing concerns about the risk of developing cross-resistance and multiple-antibiotic resistance in pathogenic bacteria both in man and livestock, linked to the therapeutic and subtherapeutic uses of antibiotics in livestock and pets.

The European Union has banned all in-feed use of antibiotics from 2006 and the use of antibiotics in feed is being considered for elimination (or intense regulation) in other parts of the world. This perspective has stimulated nutritionists and feed manufacturers to search for new, safer alternatives. The primary alternatives studied include acidification of the feed by organic acids, feeding probiotic organisms and feeding prebiotic compounds.

In the 1980s, the possible potential effects of prebiotics in animal feeds was already recognised. Since then, the interest in the use of prebiotics in animal feed and pet food has resulted in extensive research activity. Mul & Perry (1994; farm and pet animals), Houdijk (1998; swine), Iji & Tivey (1998, 1999; poultry), Flickinger & Fahey (2002; pets, poultry, swine and rabbits) and Patterson & Burkholder (2003; swine) have documented the use of prebiotics in diets for farm animals and pets.

The non-digestible inulin-type fructans are found widely in many vegetable feed and food ingredients and are perhaps the best studied and documented prebiotics in domesticated animals (Flickinger *et al.* 2003*a*).

The aim of this review is to provide an overview of recent developments on the use and application of inulin-type fructans in livestock feed and pet food including effects on intake, digestion and availability of nutrients; gut microflora and morphology; immunity and health; and performance in farm animals and pets.

Application of inulin-type fructans in diets for pigs (Table 1)

Weaning is a stressful event for pigs. Under commercial conditions, weaning piglets often face nutritional, social and psychological stress. As a result, abrupt weaning is typically accompanied by low feed intake. Weaning also causes morphological and histological changes of the small intestine of pigs resulting in maldigestion and malabsorption. When feed intake increases, enterotoxaemic bacteria may proliferate causing diarrhoea. Diarrhoea frequently occurs after the weaning transition (Nabuurs, 1991). Supplementing inulin-type fructans to weaning diets may be a practical strategy to reduce weaning-related transition of intestinal microflora by supporting beneficial bacteria such as bifidobacteria and lactobacilli and thereby decreasing intestinal pathogens like *Escherichia coli*.

Patterson & Burkholder (2003) and Flickinger *et al.* (2003*a*,*b*) summarised several experiments in which different types of fructans and other prebiotics were supplied in solid feed, formula or drinking-water to pigs alone or in combination with a probiotic. The effects of inulin-type fructans were categorised by the effect on performance; availability, digestion and retention of nutrients; gut microflora; host defence; and gut integrity. Reported effects on performance of pigs varied from little to no effect (Farnworth *et al.* 1992; Howard *et al.* 1993; Olsen & Maribo, 1999), and from mixed (Houdijk *et al.* 1998) to stimulating effects (Russell *et al.* 1996; Shim & Choi, 1997; Estrada *et al.* 2001; He *et al.* 2002; SB Shim, IH Williams and MWA Verstegan, unpublished results). Supplementation of inulin-type fructans to the diet or drinking-water resulted in fewer cases of

Abbreviations: DGGE, denaturing gradient gel electrophoresis; OF, oligofructose.

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Table 1. Effect of inulin-type fructans in pigs

Reference*	Design	Type of fructan† and dosage	Observations†,‡
I	Weanling pigs fed during 28 d	OF 3 g/kg	OF supplementation ↑ FI and ↑ ADG
II	Weanling pigs fed during 28 d	OF 2·5 g/kg	OF supplementation ↑ FI and ↑ ADG
III	135 weaned (28-d-old) pigs fed for 4 weeks	Control (corn, barley, SBM, whey, fat) Inulin 15 g/kg OF 15 g/kg	Lignosol was replaced by inulin (JAF) and OF. FI, ADG↔, VFA levels, bacterial counts in distal colonic digesta↔. Pigs fed inulin and OF had non-significant ↑ number total anaerobes and bifidobacteria. The manure samples had different colours depending on the diet. The average panel smell score of the manure ↔ [1·5 (control), 2·4 (inulin) and 2·1 (OF)]
IV	20 weaned pigs (36 h postpartum) fed nutritionally complete liquid diets for 15 d	OF 0, 3 g/l	Number of caecal bifidobacteria and total anaerobic flora; number of proximal colonic bifidobacteria and total anaerobic flora, caecal pH and SCFA concentrations ↔ Cell density and number of labelled cells in caecal epithelial mucosa ↑ CD, leading edge, labelled cells, proliferation zone, labelling index and cell density in proximal colonic eptithelial mucosa ↑ CD, leading edge, cell density, labelled cells, proliferation zone and labelling index in distal colonic epithelial mucosa ↑ by OF supplementation
V	16 weaned pigs (7-d-old) fed a non-medicated milk replacer and orally challenged with *Escherichia coli*	OF 0, 3 g/d	Within 36 h, 6 of 8 pigs developed symptoms of anorexia, pyrexia, dehydration and diarrhoea; 7 of 8 OF pigs showed no visible symptoms. OF supplementation tended to ↑ bifidobacteria compared to control. In OF pigs *E. coli* ↓ (numerically), clostridial populations ↔
VI	20 weaned pigs (8·8 kg) fed a corn–soya diet during 20 d	OF 0, 0·75 g/d, 1·5 g/d, 1·5 g/d + carbadox + Cu	ADG ↑ linearly ($P<0·25$) as OF addition increased. N digestion and retention ↑. The level of odour metabolites (concentration and excretion in the faeces) ↓
VII	96 weaned pigs fed solid diets during 4 weeks	2 × 2 factorial design: OF 0, 1 g/d; carbadox (Ab) 0, 50 mg/kg	Growth performance ↑ by OF supplementation
VIII	8 male cannulated pigs (85 kg BWG) individually housed in metabolic cages	Inulin 60 g/kg	Apparent ileal and faecal absorption and retention of Ca and P ↓; apparent faecal Zn absorption ↑ by inulin addition
IX	16 male cannulated pigs (90 kg BWG) individually housed in metabolic cages	Inulin 60 g/kg	Ileal and faecal N excretion as well as N retention ↔ by inulin supplementation
X	96 weaned (18-d-old) pigs fed solid feed during 4 weeks	2 × 2 factorial design: OF 0, 1 g/kg; SDAP 0, 35 g/kg	Pig performance ↔ by inclusion of OF. The diet did not affect CD in the small intestine. VH ↑ by both SDAP and OF. VH:CD ratio ↑ by OF
XI	Weaned (39-d-old) pigs fed solid feed during 29 d	OF 50 g/kg	FI ↑ and ADG ↑ by OF supplementation
XII	20 castrated male ileal cannulated (38-d-old, 10·4 kg) weaned pigs were fed solid feed	Control (cornstarch, glucose, casein) OF 10, 40 g/kg TOS 10, 40 g/kg	OF yielded ↑ propionate than TOS in ileal chyme. pH and volatile fatty acid pool of ileal chyme were influenced by type and level of dietary non-digestible oligosaccharide. The OF diets yielded ↑ caecal anaerobes, ↑ faecal pH, ↑ protein-derived SCFA and less butyric acid than the control diet
XIII	65 castrated male (9-week-old, 15·6 kg) pigs fed solid feed during 42 d	Control (corn/dextrose based diet) OF 7·5, 15 g/kg TOS 10, 20 g/kg	OF was not detected in faeces. OF and TOS supplementation resulted in significantly lower DM intake and ADG compared to the control pigs in weeks 1–3. During weeks 1–6 mean growth performance was not affected. Faecal pH was not affected, but faecal DM content was lower for OF/TOS pigs compared to control pigs

Table 1. *Continued*

Reference*	Design	Type of fructan† and dosage	Observations†,‡
XIV	41 weaned (21-d-old) cross-bred standard farm pigs were housed individually in metabolic cages and challenged orally with cholera toxin	Oral electrolyte solutions (OES) OES + OF (9·5 g/d)	Supplementation with OF resulted in accelerated recovery of bacteria perceived as beneficial while potentially slowing the recovery of pathogenic forms. OF did not cause an obvious reduction in the duration of diarrhoea and the associated loss of water
XV	Expt 1: 25 (57-d-old, 16 kg) pigs were fed solid feed as a slurry	Control (corn/dextrose based diet) OF 7·5, 15 g/kg TOS 10, 20 g/kg	Apparent faecal and apparent ileal nutrient digestion (DM, ash, CP, EE) ↔ by supplementation of OF and TOS
	Expt 2: 20 (38-d-old, 10·4 kg) ileal-cannulated pigs were fed solid feed		OF- and TOS-fed pigs produced dose dependently less faeces than the control pigs. The apparent faecal digestion of the non-starch neutral detergent soluble carbohydrates (including non-digestible oligosaccharides) was ↑ by OF and TOS supplementation. N and mineral balances were not affected OF was nearly completely degraded pre-caecally
XVI	90 weaned (36-d-old, 7 kg) pigs during 20 d	Control Probiotic OF (3 g/d) + probiotic	OF + probiotic resulted in ↑ count of total anaerobes, aerobes, lactobacilli and bifidobacteria as well as decreased clostridia, enterobacteriaceae and *E. coli* counts in faecal samples. Present study indicates a synergistic effect of probiotic (*Lactobacillus paracasei*) and OF on faecal microflora
XVII	2 experiments with 175 group fed pigs during 4 weeks	CON: basal diet AB: virginiamycin 0·5 g/kg OF: 50 g/kg BP: beet pulp 100 g/kg COM: OF 25 g/kg, BP 50 g/kg	Average daily gains were not significantly affected by diet. Overall AB, OF and COM ↑ ADG by 16, 9 and 6 %, respectively, compared to CON in the clean nursery trial. In the dirty nursery trial, OF ↑ feed efficiency (14 %) but reduced FI (24 %). OF was associated with lower (trend) *E. coli* concentrations in ileal contents and ↑ bifidobactera in proximal colon samples OF tended to reduce the *E. coli* concentrations and ↑ bifidobacterial and total anaerobic bacteria concentrations in the distal colon of pigs
XVIII	Expt 1: *in vitro* adhesion assay	Expt 1: inulin 0·1, 1, 5 % to incubation solution	Inulin partially inhibited adhesion of F4ac^{+} coliform to villous brush border (52 % inhibition by 5 % inulin)
	Expt 2: 20 BT-seronegative, weaned pigs (28-d-old)	Expt 2: inulin 0, 50 g/kg	Inulin supplementation to the diet did not affect the primary immune response to BT immunisation. The secondary immune response after a boost at day 21 in the inulin pigs was numerically ↑
XIX	*In vitro* inoculation of intestinal tissue (jejunal and ileal sections) from 30 pigs	Expt 1 incubation solution: inulin 25 g/kg; GOS 25 g/kg; MOS 25 g/kg	Inulin (25 g/kg) resulted in non-significant decrease in association of salmonella in ileal tissue and in association of *E. coli* in jejunal tissue
		Expt 2 dietary inclusion: inulin 40 g/kg; GOS 40 g/kg	The numbers of *E. coli* in jejunal tissue and a numerical reduction of *Salmonella* spp. in ileal tissue ↓ in inulin pigs
XX	Expt 1: 40 weaned pigs (18-d-old, 6 kg) were fed solid feed for 21 d	Control diet Control diet + OF (5 g/kg), oral dose of *Bifidobacterium longum* at days 1 and 3 Control diet (C)	The treatment with OF and *B. longum* improved ADG and FE, reduced the number of faecal total anaerobes and clostridia and ↑ the number of bifidobacteria only from day 0 to 7
	Expt 2: 80 weaned pigs (18-d-old) were fed a solid diet in a 2 × 2 factorial design	C + OF (5 g/kg) C + 1×10^{7} cfu *B. longum*/g C + OF (5 g/kg) + 1×10^{7} cfu *B. longum*/g	ADG and IGF-1 ↓ by OF supplementation, while ADG and IGF-1 ↑ by administration of *B. longum*

Table 1. *Continued*

Reference*	Design	Type of fructan† and dosage	Observations†,‡
XXI	180 weaned (17-d-old) pigs during 4 weeks in 3 phases (week 1, week 2, weeks 3 and 4)	Basal diet (BD, control) BD + inulin in water (132 g/l) BD + inulin in feed (5, 2, 1 g/kg) BD + inulin in water and feed BD + antibiotic in feed	ADG and FE ↑ by supplementation of inulin in water or feed compared to the control. Pig performance ↔ by inulin in both water and feed compared to inulin in water or feed only
XXII	90 piglets (1-d-old) were fed 10 d after birth and 10 d after weaning (36 d of age) with powdered milk	C = powdered milk L = 2 g/d C + *L. paracasei* 1×10^9 cfu/g F = 2 g/d C + *L. paracasei* 1×10^9 cfu/g; + OF (3 g/d)	Supplementation of OF in combination with *L. paracasei* did not stimulate the immune system of newborn piglets compared to C or L After weaning, OF in combination with *L. paracasei* resulted in highest numbers for almost all measured immune parameters (total count of several types of white blood cells, phagocytic activity of leucocytes and neutrophils
XXIII	36 weaned pigs (25–28-d-old) from 9 litters fed	Control (maize starch, fishmeal, dextrose) SBP 10 g/kg SBP 5 g/kg + OF 2·5 g/kg	Piglets fed with SBP or SBP + OF showed a ↑ bacterial diversity (Shannon index of diversity of DGGE bands) and a more rapid stabilisation of the bacterial community in faecal samples collected per rectum compared to control pigs Amplicons related to *Ruminococcus*-like species were found in all DGGE fingerprints derived from SBP and SBP + OF pigs but not in pigs on the control diet
XXIV	*In vitro* gas production	Inulin 0, 25 g/kg	Hourly rate of gas production of the inulin diet was ↑ to 5 ml/g diet compared to < 2 ml/g diet for the control diet. Inulin *in vitro* fermentation comprised a period of less than 10 h while the fermentation of other feed components continued until about 25 h
XXV	*In vitro* gas production	Inulin (chicory, Jerusalem artichoke) 0·2 g added to 0·5 g chyme compared with other carbohydrates and antibiotic (virginiamycin)	Except for antibiotic, the fermentation with added carbohydrates resulted in ↑ total gas production and a faster maximum rate of gas production. Addition of all tested carbohydrates except xylan resulted in shorter time at which half of the asymptotic gas had been reached. The amount of ammonia decreased for all added carbohydrates and was nearly the same for the antibiotic compared to chyme only. The branched-chain ratio was significantly lower for all additives compared with chyme whereas the antibiotic led to a significant higher value
XXVI	12 castrated male ileal-cannulated pigs (20 kg) fed *ad libitum*	Control (barley–SBM diet) GOS Inulin 20 g/kg	Protein and mineral utilisation ↔ by inulin and GOS supplementation. The intestinal fermentation (ammonia and VFA concentration) was significantly changed. Inulin degradation reached 45 % at the end of the small intestine and 100 % in the faeces

* Reference: I, Nakamura (1986); II, Fukuyasu & Oshida (1986); III, Farnworth *et al.* (1992); IV, Howard *et al.* (1993); V, Bunce *et al.* (1995*b*); VI, Bunce *et al.* (1995*c*); VII, Russell *et al.* (1996); VIII, Vanhoof & De Schrijver (1996*a*); IX, Vanhoof & De Schrijver (1996*b*); X, Spencer *et al.* (1997); XI, Shim & Choi (1997); XII, Houdijk *et al.* (1997); XIII, Houdijk *et al.* (1998); XIV, Oli *et al.* (1998); XV, Houdijk *et al.* (1999); XVI, Nemcová *et al.* (1999); XVII, Klein Gebbink *et al.* (2001); XVIII, Rossi *et al.* (2001); XIX, Naughton *et al.* (2001); XX, Estrada *et al.* (2001); XXI, He *et al.* (2002); XXII, Herich *et al.* (2002); XXIII, Konstantinov *et al.* (2003); XXIV, van Leeuwen *et al.* (2003); XXV, Bauer *et al.* (2003); XXVI, De Schrijver & De Vos (2003).

† Abbreviations: BWG, body weight gain; BT, bovine thyroglobulin; OF, oligofructose; SMB, soyabean meal; TOS, trans-galactooligosaccharides; SBP, sugarbeet pulp; GOS, galactooligosaccharides; FI, feed intake, ADG, average daily gain; JAF, Jerusalem artichoke flour, VFA, volatile fatty acids; CD, crypt depth; VH, villous height; SDAP, spray-dried animal plasma; CP, crude protein; EE, (diethyl) ether extract; FE, feed efficiency; IGF-1, insulin-like growth factor-1; DGGE, denaturing gradient gel electrophoresis.

‡ ↑, increased; ↓, decreased; ↔, no change.

diarrhoea, reduced mortality and decreased number of pigs shedding the pathogen (Bunce *et al.* 1995*b*; Oli *et al.* 1998) compared to controls.

There is scarce information on the effect of inulin and oligofructose (OF) on nutrient digestion, availability and retention. Studies by Vanhoof & De Schrijver (1996*b*), Houdijk *et al.* (1999) and De Schrijver & De Vos (2003) showed that OF and inulin supplementation does not affect protein digestion and nitrogen retention. Mineral absorption and retention was not affected by inulin or OF except for Zn (Vanhoof & De Schrijver, 1996*a*; Houdijk *et al.* 1999; De Schrijver & De Vos, 2003) and Fe (De Schrijver & De Vos, 2003).

A number of studies have attempted to investigate the effect of inulin-type fructans on intestinal and faecal microbial populations and *in vitro* gut tissue association. Some studies in pigs evaluating (a limited number of) bacterial populations showed that supplementation had little effect on size and activity of microbial populations (Farnworth *et al.* 1992; Houdijk *et al.* 1997). Some studies found enhanced intestinal bifidobacteria populations (Howard *et al.* 1993; Klein Gebbink *et al.* 2001). Others reported modulation of the intestinal flora (Nemcová *et al.* 1999) and speeding up of recovery of the normal intestinal microflora following acute diarrhoea (Oli *et al.* 1998). Konstantinov *et al.* (2003) studied the changes in time of the predominant faecal bacterial community in weaning pigs that were fed diets containing inulin-type fructans and/or sugarbeet pulp using denaturing gradient gel electrophoresis (DGGE) analysis, which is used to describe the microbial diversity in complex ecosystems including the mammalian intestinal tract. Piglets fed diets containing sugarbeet pulp (10 g/kg) or inulin-type fructans + sugarbeet pulp (2·5 + 5 g/kg) showed a higher bacterial diversity and a more rapid stabilisation of the bacterial community compared with that of the animals fed the control diet (maize starch).

Recently, some experiments have also demonstrated that inulin-type fructans affect *in vitro* fermentation kinetics when used as a substrate (Houdijk, 1998; Bauer *et al.* 2003; van Leeuwen *et al.* 2003) or affects the inoculum when included in the diet (Houdijk, 1998).

Only a few studies describe the effect of inulin-type fructans on the host defence system and gut integrity. Herich *et al.* (2002) demonstrated that the combination of OF and probiotics given to pigs before and after birth increased the number of $CD4^+$ T-lymphocytes compared to the control diet.

Inulin reduced the *in vitro* association of *E. coli* to jejunal organ tissue and of *Salmonella* spp. (non-significant) to ileal tissue (Naughton *et al.* 2001). Rossi *et al.* (2001) showed that inulin reduced the *in vitro* adhesion of a pathogenic coliform to intestinal porcine mucosa. Results also suggested a systemic specific immunomodulatory effect of inulin in immunisation against bovine thyroglobulin.

Howard *et al.* (1993) concluded that OF improved the morphological and the cellular kinetics of the epithelial mucosa in the large intestine. Spencer *et al.* (1997) investigated the effect of supplementation of spray-dried animal plasma and inulin-type fructans on the morphology of the small intestine in weaned pigs. Inulin-type fructans did not affect crypt depth but did increase the villous height and villous height:crypt depth ratio.

Shim *et al.* (SB Shim, IH Williams and MWA Verstegen, unpublished results) found that OF supplementation (2·5 and 30 g/kg) for 3 weeks post-weaning (numerically) increased villous height but not crypt depth in the small intestine of pigs compared to the control. Brush border enzyme activity was not affected.

Application of inulin-type fructans in diets for poultry (Table 2)

At hatching, the gastrointestinal tract of broilers is sterile. Immediately, bacteria originating from the mother, the environment or the diet will colonise in the gastrointestinal tract. In case of mother contacts, a diverse microbial population will enter the gastrointestinal tract. As a result, after the first colonisation, bacterial species coming later in time will have greater difficulty colonising (colonisation resistance) than the initial population. Because of the strict separation of generations in broiler chickens, any bacteria from the environment might colonise (e.g. attach to intestinal binding sites or multiply faster than being removed via chyme passage) the intestinal tract. Those feed components that are resistant to enzymatic degradation, such as inulin-type fructans, serve as a substrate for bacterial activity in the intestinal lumen. The interaction between host nutrition and the intestinal microbiota has been clearly illustrated using germ-free animals. Langhout (1998) clearly showed the importance of controlling the activity of the intestinal microbiota to support gut integrity and to avoid (i) bacterial overgrowth, (ii) reduced nutrient digestibility and (iii) reduced production performance.

Feeding inulin-type fructans may be a practical strategy for controlling pathogenic bacteria in chickens. Flickinger & Fahey (2002) and Flickinger *et al.* (2003*a*) summarised several experiments in which different types of fructans were fed to broilers alone or in combination with a probiotic to evaluate the effect on colonisation of pathogens (i.e. *Salmonella* spp. and *Campylobacter jejuni*) in caeca (Bailey *et al.* 1991; Oyarzabal & Conner, 1996; Chambers *et al.* 1997; Fukata *et al.* 1999) and on prechilled poultry carcasses. Researchers concluded that supplementation of inulin-type fructans in combination with competitive exclusion flora may reduce colonisation by the pathogenic bacteria.

In recent experiments with broilers, we (Verdonk & van Leeuwen, 2004) evaluated the effect of supplementation of inulin-type fructans on the colonisation and shedding of pathogens. The first broiler study evaluated the effect of the inclusion of 20 g OF and inulin/kg feed on the colonisation and shedding of *Salmonella typhimurium* and *C. jejuni* in broilers. The broilers were fed one of four dietary treatments and challenged in the crop on days 10 and 11 of age with a low or high dose of *S. typhimurium* and a single dose of *C. jejuni*. The birds were housed in three-tier battery cages. Feed intake and body weight were measured at ages 9, 14, 21 and 35 d. During dissection of birds on days 14, 21 and 35, digesta samples of the crop and caeca were taken and the colonisation was determined. On days 18 and 28, salmonella shedding via the excreta was measured.

The second study evaluated the effect of inulin at four inclusion levels in a basal diet on the occurrence of lesions due to *Eimeria acervulina* and *Clostridium perfringens*. The broilers were housed in floor pens and given an *E. acervulina* challenge orally at day 10 of age, followed by an oral inoculation of *Cl. perfringens* on days 14, 15 and 16. Intestinal lesions for coccidiosis and necrotic enteritis in the duodenum and jejunum on days 15–17 and 22 were scored visually.

The supplementation of inulin-type fructans in the diet stimulated the performance of young broiler chickens, but did not clearly affect the colonisation and shedding of *S. typhimurium* and *C. jejuni* or the occurrence of lesions due to *E. acervulina* and *Cl. perfringens*.

Table 2. Effect of inulin-type fructans in poultry

Reference*	Design	Type of fructan† and dosage	Observations†,‡
I	2800, 1-d-old male broilers fed for 46 d	Commercial diet OF in feed (2·5, 5·0 g/kg) BMD	Final body weights numerically ↑ and FE ↑ by OF (results are not mentioned in the abstract but are referred to in Flickinger *et al.* 2003*a*,*b*)
II	2400, 1-d-old male broilers fed for 47 d	Control OF 3·75, 7·5 g/kg Antibiotic (virginiamycin) 0·011 g/kg	Supplementation of OF at the inclusion level of 3·75 resulted in heavier birds and improved carcass yield (hot carcass weight, percentage breast meat) at 47 d compared to control and antibiotic groups
III	Broilers	Control OF 3·75 g/kg	ADG, FE and carcass quality ↔ by OF supplementation
IV	10 pens with salmonella-negative day-old Cornish broiler chicks	Control Inulin crude Inulin refined Lactulose Lactosucrose	Salmonella score ↔ by supplementation of carbohydrates Salmonella counts at 6 weeks in broilers fed crude inulin ↑ compared to all other broiler groups. Infections ↓ in broilers fed refined inulin than in control broilers. The decline of salmonella infection in broilers fed refined inulin ceased after the dietary additive was discontinued at 5 weeks of age. Caecal pH and density ↓ by inulin
V	880 Ross broiler chicks in 16 pens, challenged at day 2 after hatching with *Salmonella typhimurium* by spraying	Control: buffered peptone water OF + microbial mixture	Treatments were provided in drinking-water at 6 weeks of age after feed with drawl. Caeca from treated birds weighed more than from control birds. No difference in salmonella colonisation occurred between the treated and control group
VI	84, 1-d-old Hubbard × Hubbard male broilers were housed in battery pens and fed a corn–soyabean diet with sprayed kestose/sugars for 28 d	Control (C) C + 100 g kestoses (OF), sugars/kg C + 80 g sugars/kg	ADG, FE or concentrations of total aerobic bacteria, coliforms, lactobacilli, total anaerobes or clostridia ↔ by supplementation of kestoses. Caecal bifidobacterial populations ↑ 24-fold in kestose-treated birds
VII	Expt 1 and 2: two trials with 60, 1-d-old birds (White Leghorn Hy-Line) challenged orally with *Salmonella enteritidis* at day 7 (expt 1) or 21 (expt 2)	Control CE OF (1 g/kg) CE + OF (1 g/kg)	Did not effect caecal salmonella colonisation or translocation in birds challenged at 7 d ↔ by OF supplementation. In birds challenged at day 21 the mean numbers of *S. enteritidis* in caecal contents on days 1 and 7 post-infection ↓ by OF supplementation or OF + CE
	Expt 3 and 4: a total of 20, 1-d-old birds	Control CE OF (1 g/kg) CE + OF (1 g/kg)	The major bacterial population of the caecal microflora ↔ by OF supplementation
VIII	24 ISA Brown laying hens (20 weeks old) housed individually	Wheat based Oat based Millrun based Rice hull based Wheat + OF (2 g/kg) Wheat + MOS (2 g/kg)	Gut viscosity and egg production ↔ by dietary treatment
IX	312, 1-d-old male Cobb broiler chicks in 26 pens during 35 d	Control OF Glucose MOS	Supplementation of glucose, OF or MOS tended to result in heavier birds with better FE until day 35

Table 2. *Continued*

Reference*	Design	Type of fructan† and dosage	Observations†,‡
X	98, 1-d-old male and female Ross broiler chicks in 12 pens	Control corn–soya Inulin 10 g/kg OF 10 g/kg	Faecal microbial counts of total aerobe, lactobacilli, salmonella and campylobacter at 2, 4 and 6 week of age ↔ by the dietary treatment. Total faecal aerobes and *Escherichia coli* at week 4 ↓ by OF compared to the control Lactobacilli counts in the gizzard and small intestine ↑ in female OF birds. Total campylobacter counts in the large intestine ↓ in inulin and OF birds Faecal ammonia content and pH during weeks 1–4 ↓ in OF birds but not in inulin birds
XI	98, 1-d-old male and female Ross broiler chicks in 12 pens	Control corn–soya Inulin 10 g/kg OF 10 g/kg	ADG, carcass weight, FE and gut length in female birds ↑ but in male birds ↔ by OF and inulin supplementation
XII	Expt 1: 864, 1-d-old male Ross 308 birds were fed during 35 d and orally challenged with salmonella and campylobacter	OF 20 g/kg Inulin 20 g/kg High-molecular-inulin 20 g/kg	Performance of young broiler chickens ↑ Colonisation and sheding of salmonella and campylobacter ↔
	Expt 2: 704, 1-d-old male Ross 308 birds were housed in 32 floor pens and orally challenged with *Eimeria acervulina* and *Clostridium perfringens*	Inulin	Performance of young broiler chickens ↑ Occurrence of lesions due to *E. acervulina* and *Cl. perfringens* ↔

*Reference: I, Ammerman *et al.* (1988); II, Ammerman *et al.* (1989); III, Waldroup *et al.* (1993); IV, Chambers *et al.* (1997); V, Oyarzabal & Conner (1996); VI, Patterson *et al.* (1997); VII, Fukata *et al.* (1999); VIII, Hartini *et al.* (2003); IX, Ao & Choct (2003); X, Yusrizal & Chen (2003*a*); XI, Yusrizal & Chen (2003*b*); XII, Verdonk & van Leeuwen (2004).

† Abbreviations: OF, oligofructose; BMD, bacitracin methylene disalicylate; CE, competitive exclusion; MOS, mannan oligosaccharides; FE, feed efficiency; ADG, average daily gain.

‡ ↑, increased; ↓, decreased; ↔, no change.

Yusrizal & Chen (2003*a*) reported that supplementation of OF and inulin (10 g/kg) to a corn–soya diet did not affect the faecal microbial counts of total aerobe, lactobacilli, salmonella and campylobacter in broilers at 2, 4 and 6 weeks of age. OF resulted in significant reductions in total faecal aerobes and *E. coli* at week 4 compared to the control. It also increased the lactobacilli counts in the gizzard and small intestine of female broilers. Inulin or OF supplementation reduced total campylobacter counts in the large intestine. OF but not inulin resulted in reduced faecal ammonia content and pH during weeks 1 through 4. The same authors (Yusrizal & Chen, 2003*b*) reported that supplementation of OF and inulin improved body weight gain, carcass weight, feed conversion efficiency and gut length in female birds but not in male birds. Also, Ao & Choct (2003) reported that birds given drinking-water supplemented with OF (0·05 %) were heavier and had more efficient feed conversion at day 35 compared to the control birds. Recently, Hartini *et al.* (2003) concluded that supplementation of inulin-type fructans (2 g/kg) to a wheat-based diet did not affect the feed intake nor egg production in ISA Brown laying hens.

Application of inulin-type fructans in diets for (preruminant) calves (Table 3)

The common practice of early weaning of preruminant calves for veal production, followed by long distance transport and regrouping of animals from different origins, may cause a challenge to the natural defence system resulting in dysbacteriosis and digestive disorders. In many fattening systems, starter treatments with antibiotics have become common practice. Mul & Perry (1994) mentioned a large-scale use of inulin-type fructans resulting in similar fattening results compared to the in-feed antibiotics. Verdonk & van Leeuwen (2004) have evaluated the effect of inclusion of inulin-type fructans in the milk replacer on health and production performance of preruminant calves during the first 3 weeks after arrival at the fattening farm. Four groups of eight calves, housed individually in wooden boxes with a slatted floor, were fed the basal calf milk replacer supplemented with 20 g of dextrose, OF, inulin or dextrose supplemented with antibiotics/kg. Individual body weight of the calves was determined at 7 d intervals and feed intake was measured per calf per feeding. The faecal consistency scores were conducted daily. The composition of the microflora in rectal samples was determined by DGGE and nucleotide sequence analysis of rDNA. During the 3-week experimental period, the OF and the antibiotics groups resulted in higher ($P<0·05$) body weight gain compared to the dextrose group. The faeces consistency over the observed period was best ($P>0·05$) in the OF group and worse in the dextrose group. The DGGE gels revealed that the faecal flora in young milk-fed calves in fattening farms is very unstable. The dietary treatment did not affect the pattern or the shift in pattern of the bands in the gels of the faecal samples. Analysis of ileal contents and faeces showed that some 70 % of the dietary inulin-type fructans reached the caecum but that no fructans were recovered in the faeces.

Kaufhold *et al.* (2000) supplemented 10-week-old calves (average body weight 117 kg) with 0 or 10 g OF/d (with the morning meal). Feed intake was similar between groups but weight gain tended to be higher for the OF-supplemented group. They concluded that OF had basically similar effects on the metabolic and endocrine traits such as concentration of glucose, lactate, triacylglycerols and insulin in blood in preruminant calves as in animals and human subjects with diabetes mellitus.

Webb *et al.* (1992) observed greater weight gains in Holstein bull calves (3–5 d old) by adding a combination of inulin-type fructans (3·75 g/kg), sodium diacetate (10 g/kg) and decoquinate (50 mg/kg) into the milk replacer and starter grain compared with supplementing the milk replacer with sodium diacetate and decoquinate alone. Unfortunately, the effects on rumen and gut microflora were not studied.

Donovan *et al.* (2002) reported that supplementation of a blend of inulin-type fructans, allicin and gut-active microbes to the milk replacer had similar effects to those of the antibiotics-supplementation fed to Holstein male and female calves.

Compared with diets for other species, dietary proteins and carbohydrates for veal calves are usually highly digestible. This was to a large extent related to the soluble/dispersible nature of the proteins used in veal calf diets. Commercial milk replacers were initially made based primarily on skimmed milk powder and animal fat. During the last decade, replacement of milk proteins and lactose by vegetable proteins and carbohydrates has become an important issue both in practice and research (Verdonk *et al.* 2002). Increasingly, part of the dietary lactose is being replaced by starch and by soya oligosaccharides. Up to 15 % starch can be added to veal calves' diets, with only a minor decrease in starch digestibility. At higher levels (15–25 %), the decrease in starch digestibility is more pronounced (van Weerden *et al.* 1967; van der Honing *et al.* 1974) and this causes increased fermentation in the large gut. Visual characteristics and pH of the faeces were affected by the quantity of starch fermented in the hindgut. We have demonstrated (Verdonk *et al.* 1998) that replacement of lactose (65 g/kg) by soluble or insoluble soya carbohydrates resulted in significantly decreased apparent ileal digestibility of DM, crude ash and N-free extract. Inclusion of the soya carbohydrates in the diet also tended to increase the endogenous flow of N at the terminal ileum. It was suggested that this increase might be caused by fermentation in the small intestine increasing the flow of bacterial N to the large gut. Inulin-type fructans may play a role in creating and maintaining a desired, stable microflora in the rumen (supplementation to solid feed), and the small and large gut (supplementation to milk replacer) of (preruminant) calves.

Application of inulin-type fructans in pet foods (Table 4)

Several reasons that justify the addition of OF and inulin in pet foods are as follows (Flickinger *et al.* 2003*a*):

- manipulating the composition of the intestinal flora,
- stimulating gut integrity,
- affecting nitrogen metabolism, and
- reducing offensive faecal odour.

Furthermore, it was indicated that the geriatric pet population is more prone to intestinal irregularities and has diminished digestive microbial balance when compared to younger animals. In their review, Flickinger *et al.* (2003*a*) summarise the results of studies indicating an effect of supplementation of inulin-type fructans on the intestinal microflora, epithelial cell proliferation, faecal characteristics and nutrient digestibilities.

Recently, Hesta *et al.* (2001) studied the effect of supplementation of OF (30, 60, 90 g/kg) and inulin (30, 60 g/kg) to a com-

Table 3. Effect of inulin-type fructans in calves

Reference*	Design	Type of fructan† and dosage	Observations†,‡
I	40 male Holstein (3–5-d-old) fed milk replacer (MR) and calf starter (CS) during a 10-week period	OF 0, 3·75 g/kg in MR and 0, 8·8 g/kg in CS	In winter time, ADG ↑ by adding a combination of OF, sodium diacetate and decoquinate compared with controls. OF source NR
II	Veal calves in practice	OF + probiotic (5/2·5 g/kg 0–6 week/7–26 week)	Authors mention unpublished results in practice (Italy, Netherlands) showing similar fattening results comparing a feeding regime using OF compared to antibiotics
III	21 calves fed milk replacer and calf starter	0, 3, 7 g OF/d	Plate counts in faecal samples for bifidobacteria ↑ ($P=0·02$) by OF supplementation to the milk replacer. Counts for *Escherichia coli*, clostridia and the total anaerobes were numerically changed
IV	14 Simmental × Red Holstein calves (10 weeks old) fed whole milk plus milk replacer during a 3-week period	GrC = no supplementation GrF = 10 g OF/d	ADG tended to be higher for GrF. The postprandial increase of glucose concentrations was significantly smaller, of lactate tended to be smaller, whereas maximal insulin concentrations reached were significantly higher in OF calves
V	45 male and female Holstein calves (newborn) fed milk replacer and calf starter	MRA = antibiotics in milk replacer MRE = OF + allicin + probiotics	Total weight gain, feacal scores and serum proteins during 5-week experimental period was not different between groups. OF source NR
VI	32 male black and white (1-week-old) calves fed milk replacer for 3 weeks	Dextrose (DEX), OF (20 g/kg) Inulin (20 g/kg) Dextrose + antibiotics (ANT): 20 g/kg	ADG ↑ for OF and ANT calves compared to DEX, faecal score numerically better for inulin compared to DEX Composition of faecal microflora ↔ and highly variable

* Reference: I, Webb *et al.* (1992); II, Mul & Perry (1994); III, Bunce *et al.* (1995*a*); IV, Kaufhold *et al.* (2000); V, Donovan *et al.* (2002); VI, Verdonk & van Leeuwen (2004).
† Abbreviations: OF, oligofructose; ADG, average daily gain; NR, not reported.
‡ ↑, increased; ↓, decreased; ↔, no change.

Table 4. Effect of inulin-type fructans in pets

Reference*	Design	Type of fructan† and dosage	Observations†,‡
I	16 IgA-deficient 15- to 20-month-old German Shepherd dogs with intestinal bacterial overgrowth were fed a chicken based kibble diet for 50 d	OF 10 g/kg	Number of aerobic bacteria in digesta from the duodenum/proximal part of jejunum and in the duodenal mucosa ↓ by supplementation with OF
II	*In vitro* fermentation using cat faecal inoculum. Cats were fed a diet with or without supplemental fibre (beet pulp)	OF	OF together with some fermentable fibre sources resulted in highest OM disappearance and acetate, propionate and total SCFA production
III	*In vitro* fermentation using dog faecal inoculum	OF	OF had greatest OM disappearance ($P<0{\cdot}05$; >80 %), greatest propionate and intermediate total SCFA production
IV	8 castrated 1- to 1·4-year-old young adult male beagles were fed minced meat, flaked maize based diet for 42 d	Control (C) C + OF 40 g/kg + SBF 10 g/kg C + OF 80 g/kg + SBF 20 g/kg	Total-tract digestibility of CP ↓ by OF, but digestibility of DM, OM and EE ↔. Postprandial plasma concentrations of insulin and cholesterol ↔
V	8 beagle dogs (2 intact males and 6 neutered females), 5 years old, were fed minced one of four meat, flaked maize based diet in a 4 × 4 latin square design for 20 weeks	Control (C) C + inulin 70 g/kg DM C + SBF 70 g/kg DM C + guar gum 70 g/kg DM	Inulin increased wet faecal output and water consumption compared to C. Supplementation of inulin decreased digestibility of OM, CP and EE. Inulin showed no metabolic effects
VI	6 male and 6 female healthy specific pathogen-free cats were fed a dry diet for 32 weeks	OF 7·5 g/kg	Wide quantitative and qualitative variation in the duodenal flora was observed over time. Duodenal flora ↔ by OF
VII	6 male and 6 female healthy specific pathogen-free cats were fed a dry diet for 32 weeks	OF 7·5 g/kg	Supplementation with OF resulted in alteration of faecal flora of cats. Total bacterial counts, aerobic and anaerobic counts were not affected by diet. Mean counts of *Lactobacilli* and *Bacteroides* spp. ↑ and *Escherichia coli* and *Clostridium perfringens* ↓ by OF
VIII	10 adult beagle dogs of both sexes were fed a chicken (by) product brewers rice based diet for 6 weeks	Cellulose 36 g/kg Beet pulp + OF (42 + 10 g/kg)	Small intestinal dimensions ↑ and *in vitro* carrier-mediated glucose uptake ↑ by fermentable beet pulp and OF
IX	Expt 1: 8 healthy adult cats, basal diet containing 26·5 g/kg crude fibre	OF 0, 30, 60 and 90 g/kg	Supplementation with 60 and 90 g OF/kg significantly affected faecal characteristics
	Expt 2: 8 healthy adult cats, same basal diet, faeces collection	OF 30 g/kg Inulin 0, 30, 60 g/kg	OF and inulin resulted in lower apparent faecal protein digestibility
X	7 adult mixed breed female dogs in a 4 × 7 incomplete latin square design	Crude inulin, 5 g/kg	Ileal, large intestinal and total-tract DM and N digestibility ↔, minor effects on bacterial growth in the large intestine and VFA proportions in the small intestine
XI	*In vitro*	OF, 4 inulin products	Total production of SCFA ↑ for OF and the inulin products OF, DP=3–5; inulin HP, DP = 9; inulin IQ DP=9; inulin, DP>12; OF, DP=2–8 compared to beet pulp, MOS and soya fibre or cellulose
XII	5 healthy dogs in a cross-over trial	OF 10 g/kg	OF (DP=3–20) ingestion changed faecal microflora, apparent Mg and Ca absorption ↑. Faecal pH and the route of N excretion ↔. Diet used in the present study contained rice, corn and beet pulp and probably was rich in non-digestible fermentable carbohydrates
XIII	Expt 1: 20 healthy female and male dogs	OF, *Lactobacillus acidophilus* (LAC)	In expt 1, *Cl. perfringens* ↓ ($P=0{\cdot}08$) and faecal butyrate ($P=0{\cdot}06$) and lactate ($P<0{\cdot}05$) concentrations ↑ by OF
	Expt 2: 20 healthy female and male dogs	OF + LAC 6·7 g/kg (or 2 g OF/d), 1×10^9 cfu, 6·7 g/kg OF + 1×10^9 cfu	In expt 2, FI and faecal DM output tended ↓ OF had lowest faecal concentrations of hydrogen sulfide, methanethiol and dimethyl sulfide. LAC was more affective when fed in combination with OF

Table 4. *Continued*

Reference*	Design	Type of fructan† and dosage	Observations†,‡
XIV and XV	4 adult female dogs surgically fitted with ileal cannulas	OF, MOS, OF + MOS 5, 5, 10 g/kg (or 2 g OF/d)	Ileal IgA concentrations ↑ in OF + MOS dogs, faecal microbial populations ↔ by OF Faecal total indole and phenol concentrations ↓ by OF and OF + MOS. The dose of 2 g OF/d was maybe not high enough to affect the microbial population in the distal colon or faeces
XVI	Expt 1: 16 adult male beagles (12 kg, 3 years old)	OF 0, 3, 6, 9 g/kg (or 0, 0·6, 1·2, 1·8 g OF/d)	Total-tract digestibility of DM ($P<0·05$), OM ($P<0·05$), lipid ($P<0·01$) and CP ($P<0·07$) ↓ Stool quality ↔ Faecal ammonia and urinary ammonia concentration numerically ↓ by 45 % Faecal concentrations of propionate ($P<0·05$), butyrate ($P=0·15$) and total SCFA ($P=0·07$) ↑ in OF supplemented dogs
	Expt 2: 4 adult female dogs (20 kg, 3 years old), surgically fitted with ileal cannulas	OF 0, 2, 4, 6 g/kg (or 1, 2, 3 g OF/d)	Faecal odour components and total anaerobe concentration ↔. Total aerobe concentration tended ↓ Ileal nutrient digestibility numerically ↑ with increasing OF. Faecal concentrations of SCFA, BCFA, ammonia, phenols and indoles ↔. Faecal total aerobes ↑ and *Cl. perfringens* ↓
XVII	7 ileal-cannulated adult female dogs, 7 × 7 latin square	OF 0, 3, 6, 9 g/kg Inulin 0, 3, 6, 9 g/kg	Nutrient intake and ileal digestibility ↔ Total-tract digestibility of DM, OM and CP ↓ Faecal ammonia and SCFA ($P<0·10$) concentrations ↑ Faecal total phenols ↓ Individual and total amines (only by OF) ↓ by supplementation of OF and inulin

* Reference: I, Willard *et al.* (1994); II, Sunvold *et al.* (1995*a*); III, Sunvold *et al.* (1995*b*); IV, Diez *et al.* (1997); V, Diez *et al.* (1998); VI, Sparkes *et al.* (1998*a*); VII, Sparkes *et al.* (1998*b*); VIII, Buddington *et al.* (1999); IX, Hesta *et al.* (2001); X, Strickling *et al.* (2000); XI, Vickers *et al.* (2001); XII Beynen *et al.* (2002); XIII, Swanson *et al.* (2002*a*); XIV, Swanson *et al.* (2002*c*); XV, Swanson *et al.* (2002*b*); XVI, Flickinger *et al.* (2003*b*); XVII, Propst *et al.* (2003).

† Abbreviations: OF, oligofructose; SBF, soyabean fibre; MOS, mannan oligosaccharides; OM, organic matter; CP, crude protein; EE, (diethyl) ether extract; VFA, volatile fatty acids; DP, degree of polymerisation; BCFA, branched-chain fatty acids.

‡ ↑, increased; ↓, decreased; ↔, no change.

mercial diet in cats. Supplementation of 60 and 90 g/kg OF in the diet significantly affected faecal characteristics. Both OF (30 g/kg) and inulin (30, 60 g/kg) resulted in lower apparent faecal protein digestibility. These results contradict Diez *et al.* (1997), who reported that 40 or 80 g diet OF/kg did not reduce total-tract digestibility of DM, organic matter or ether extract for dogs fed a beef-, corn- and vegetable oil-based diet. However, Diez *et al.* did report that supplementation with 80 g OF/kg reduced the digestibility of crude protein for supplemented diets compared to the control diet.

At lower inclusion levels of inulin-type fructans (1–10 g/kg), results on nutrient digestibility in dogs are conflicting and range from (i) no effect on ileal and total-tract nutrient digestibility (Strickling *et al.* 2000; Beynen *et al.* 2002; Grieshop *et al.* 2002; Swanson *et al.* 2002*b*; Propst *et al.* 2003), (ii) a decreased total-tract nutrient digestibility (Flickinger *et al.* 2003*b*; Propst *et al.* 2003) and (iii) an increased absorption of Mg and Ca (Beynen *et al.* 2002).

Conclusions

Important issues for pet owners and farmers are (1) animal health and veterinary costs, (2) performance and economics and (3) excretion of nutrients into the environment. Inulin-type fructans may play a role in solving these issues. There are many considerations in supplementing inulin-type fructans in animal feed and pet food. These include the type of diet (i.e. the content of non-digestible oligosaccharides); the type and inclusion level to supplement; the animal characteristics (species, age, stage of production); and the hygienic conditions of the farm.

The number of studies evaluating the potential of inulin-type fructans in animal feed and pet food has increased considerably during the last few years. Studies indicate a generally positive effect on gut microflora, host health (gut integrity, colonisation) and animal performance (digestion, body weight gain, feed efficiency). However, the data on the efficacy of inulin-type fructans are sometimes variable and not yet fully conclusive. Data on the effect of inulin-type fructans on intestinal and systemic immune systems as well as the resistance to infections remain scarce.

Costs of animal production will increase when in-feed antibiotics are banned; thus, there is a need for conclusive data to determine under which conditions inulin-type fructans can reduce the impact of (sub-clinical) infections and support animal performance.

There is a need for more standardised studies using both negative and positive controls to study the efficacy of inulin-type fructans. The control groups as well as the experimental setting should be chosen in line with the selected objective of the study; for example, animal performance or nutrient excretion. Both technical and economic parameters should be evaluated to be able to conclude on the effectiveness of fructan supplementation.

In order to effectively supplement inulin-type fructans in feed and pet food, additional research is also needed to elucidate the mode of action and the relationship between gut microflora, gut and animal health, and performance. Molecular DNA techniques might be helpful in future research to gain further insight into the changes occurring in the composition of the gut microflora and the gene expression in gut tissue and relevant organs.

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