Universitat Autònoma de Barcelona
Departament de Ciència Animal i dels Aliments

WHEAT BRAN MODIFIES THE MICROBIAL POPULATION AND ENHANCES GUT FERMENTATION IN THE GASTROINTESTINAL TRACT OF POST-WEANING PIGLETS

MEMÒRIA PRESENTADA PER FRANCESC MOLIST GASA PER ACCEDIR AL GRAU DE DOCTOR DINS DEL PROGRAMA DE DOCTORAT DE PRODUCCIÓ ANIMAL DEL DEPARTAMENT DE CIÈNCIA ANIMAL I DELS ALIMENTS

BELLATERRA, 2010

FACULTAT DE VETERINÀRIA DE BARCELONA

José Francisco Pérez Hernández, professor titular del departament de Ciència Animal i dels Aliments de la Facultat de Veterinària de la Universitat Autònoma de Barcelona i Arantza Gómez de Segura Ugalde, investigadora contractada del departamento de Nutrición, Bromatología y Tecnología de los alimentos de la Facultad de Veterinària de la Universidad Complutense de Madrid.

certifiquen:

Que la memòria titulada "Wheat bran modifies the microbial population and enhances gut fermentation in the gastrointestinal tract of post-weaning piglets", presentada per Francesc Molist Gasa per optar al grau de Doctor en Veterinària amb menció europea, ha estat realitzada sota la seva direcció i, considerant-la acabada, autoritzen la seva presentació per que sigui jutjada per la comissió corresponent.

I per que consti als efectes oportuns, signen la present a Bellaterra, 19 de Maig de 2010.

Dr. José Francisco Pérez Hernández Dra. Arantza Gómez de Segura Ugalde

The work has been financed by the project AGL2005-07438-C02-01. The author was in receipt of a grant from the Ministerio de Educación y Ciencia (Spain).

La defensa d'una tesis, és per a mi la culminació d'una nova etapa en la meva vida. Crec que per això és important dedicar les primeres pàgines d'aquesta memòria a totes les persones que durant aquest temps m'han ajudat a arribar fins aquí.

Començant pels meus inicis, abans fins i tot de la meva etapa universitària, m'agradaria donar les gràcies als meus avis per transemetre'm la passió pels animals i introduir-me en l'ofici de veterinari. Al mateix temps també m'agradaria reconèixer l'esforç fet pels meus pares i el seu suport rebut durant tot aquest temps. M'agradaria continuar donant gràcies a la resta de la meva família per empenyem un cop acabada la carrera, a endinsar-me en els estudis de doctorat.

Un cop presa la decisió de començar els estudis de la tesis també voldria reconèixer el treball d'en Francisco. Primer de tot per escriure el projecte amb el qual he treballat durant tot aquest temps, en segon lloc per oferir-me la possibilitat de realitzar la tesis doctoral amb la obtenció de la beca i finalment per ajudar-me a créixer i a formar-me així com aprendre com es pot viure amb passió el treball diari. Cal reconèixer que tot això tampoc hauria estat possible si el senyor ZP no m'hagués ajudat, encara que fos amb una mòdica quantitat econòmica, durant tot aquest temps. Lògicament també vull agrair el suport i l'ajuda rebut de tot l'equip docent i investigador del Grup de: Josep, Mariola, AnaCris, Roser, Susana i Olga. També foren d'especial ajuda en el primer any els consells de totes aquelles becàries (i algun becari) amb àmplia experiència en el camp de l'investigació i les tesis com l'Edgar, la Montse, l'Eva, l'Alba, la Marisol, en Gabri, en José, en Juan Carlos, la Marta, la Sandra o la Walkiria. Un paràgraf especial es mereix la meva co-directora de tesis i amiga: l'Arantza. Tot i la seva escassa experiència en el camp de la nutrició porcina, però àmplia formació en l'àrea química, ha estat un complement perfecte d'en Francisco i crec que al final formarem un gran grup, quasi una família. Digne de reconèixer ha estat la seva tenacitat, esforç i esperit de superació. Un model a seguir.

Aquests anys de treball en la tesis, també m'han permès fer estades en centres estrangers per adquirir nous coneixements i viure noves experiències és per això que m'agradaria també recordar la gent amb la que m'he creuat en cada un d'aquests viatges. De la primera estada a Finlàndia, vull donar les gràcies al

professor Timo Korhonen i la Dra. Ritva Virkola així com el seu equip per obrir-me les portes del seu departament i ajudar-me en el meu projecte. També vull donar gràcies a l'Ossi i a l'Anna per fer que la meva experiència en terres nòrdiques fos inoblidable. "Suomen oleskeluuni liittyen haluan erityisesti kiittää professori Timo Korhonen ja tohtori Ritva Virkola työrymineen, jotka avasivat minulle yksikkönsä ovet ja auttoivat minua kehittämään tutkimusprojektini työskentelyä. Kiitos myös Ossi Lehtiselle ja Ana Rodriguezille, jotka mahdollistivat unohtumattoman kokemukseni pohjoismaassa".

De la segona estada a Canadà, també voldria dedicar unes paraules a tota la gent que tant amablement em va acollir a Winnipeg. Pimer de tot gràcies al professor Martin Nyachoti per oferir-me la possibilitat de treballar amb el seu grup i col·laborar amb el desenvolupament de la tesis. També vull agrair l'esforç d'en Sanjiv i la calidesa d'en Juan David, en Gustavo, en Giancarlo i el professor Rodríguez. "Thank you very much professor Martin for giving me the opportunity to come and work with you in my thesis project. At the same time I also want to say thank you to Sanjiv and your entire group for their help and cooperation". Del penúltim any m'agradaria recordar l'experiència viscuda a la magnífica ciutat de Kyoto. Moltes gràcies al professor Ushida i Dr. Ryo per oferir-me la possibilitat d'estar entre vosaltres i viure una experiència inoblidable amb tots els estudiants del vostre departament: Tohru, Yuko, Sho, Kenichi, Saori, Ippei, Saeko, Taka, Tomoko, Hiro, i Asami. També m'agradaria recordar l'altre visió del Japó que em van donar la Anri i enYuichi."日本を訪れ、そこで働く機会を与えてくださった牛田教授と井上講師に感謝いたします。新たな文化を学んだり素晴らしい人々（徹、祐子、祥、謙一、さお里、一平、紗英子、崇之、智子、大典、亜沙美）に出会えたりしたこと全てに感謝いたします。私にとって本当に素晴らしい経験となりました。"

Més recentment i com a última estada de final de doctorat també vull tenir unes paraules de reconeixement per totes les persones conegudes a Nova Zelanda. En especial al professor Moughan i en Shane pel seu esperit de treball, en Carlos per els bons moments viscuts i a tota la comunitat del Riddet Institute així com els meus companys de casa per la gratificant experiència viscuda. "Thank you very much to professor Moughan and Mr. Shane for their help and their spirit of work, thank's to

Carlos for the nice experience that we had and thank you very much to the multicultural community of the Riddet Institute as well as my housemates for the rewarding experience".

També m'agradaria fer un reconeixement als nou vinguts com en David o la Lorena, la Rosa, en Cayo, la Sabrina, la Mara, en Mauro, en Houssein o la Luiza entre altres; així com tots els becaris que han iniciat la seva carrera doctoral i als quals els dono tots els meus ànims i forces perquè arribin fins aquí. Molta sort i moltes gràcies per la seva ajuda amb en Rafael, la Elisa, en Ramon, la Gemma, l'Alexei, en Jaime, en Piero, en Víctor, en Roger i la Clara.

També crec que es mereixen un recordatori el Servei de Granges, el SAQ, el Servei de Qualitat o el la gent del CRESA per la seva col·laboració i ajuda.

Part de l'ajuda per arribar fins aquí també l'he rebut dels meus companys de Manlleu, molt especialment als que hem passat bons moments jugant a bàsquet així com els companys de la universitat que després d'acabar la carrera encara mantenim l'amistat per seguir vivint bones experiències.

Per acabar amb els reconeixements vull tornar a donar les gràcies per tot el suport rebut per part de la meva família: els meus pares, la meva germana, els meus tiets i cosins i finalment a la Mireia que m'ha aportat estabilitat i m'ha fet costat en totes les decisions preses. Només em queda desitjar que la pròxima etapa que ara començo la pugui seguir compartint amb tots vosaltres.

RESUM

L'objectiu de la present tesis fou estudiar si la incorporació d'ingredients fibrosos a la dieta de garrins recent deslletats, era una bona estratègia per minimitzar els desordres intestinals que normalment ocorren durant l'etapa post-deslletament, i d'aquesta manera facilitar l'adaptació digestiva dels animals en les següents etapes de creixement.

Per aconseguir aquest objectiu, es dissenyaren quatre proves (capítols 4 a 7) experimentals.

En la Prova 1 (Molist et al., 2009a), primer de tot volíem confirmar uns resultats preliminars obtinguts en una prova anterior on s'havia observat un major creixement dels animals quan una font de fibra insoluble (segó de blat, WB) fou introduïda en una dieta de garrins post-deslletament. Al mateix temps, també volíem analitzar si aquest tipus de fibra insoluble era adequada per aquest període de creixement dels animals, o si per contra, era més interessant incorporar un tipus de fibra soluble (com la polpa de remolatxa, SBP). L'objectiu de l'estudi era explorar l'efecte d'incloure dos tipus diferents de fonts de fibra (WB, insoluble i SBP, soluble) sobre el creixement, les característiques físico-químiques de la digesta i l'activitat metabòlica i la composició de la microbiota intestinal. Els resultats mostraren que la fermentació intestinal fou baixa durant la primera setmana post-deslletament. L'addició de WB o WB i SBP en la dieta incrementaren la fermentació intestinal i la concentració d'àcid butíric en la digesta cecal juntament amb una reducció de la població d'enterobactèries en les femtes. La conclusió de l'estudi fou que el consum d'un tipus de fibra insoluble durant els primers dies després del deslletament (ja sigui WB o WB-SBP) modifica les característiques físico-químiques de la digesta i afecta la colonització microbiana a l'intestí gros. També especularem que els efectes observats amb la inclusió de WB podrien està relacionats amb: 1.- canvis en les característiques físico-químiques de la digesta, tals com una majora capacitat de retenció d'aigua (WRC) i una major fermentació de la digesta intestinal, 2.- un efecte físic relacionat amb la mida de partícula gran o 3.- una reducció del temps de trànsit de la digesta intestinal.

En la Prova 2 (Molist et al., 2009b), es volia confirmar la reducció de la població d'enterobactèries promoguda pel WB, i la seva capacitat per reduir els desordres digestius front a una infecció experimental amb *E. coli* K88. A més a més, es volia clarificar si aquest efecte observat amb la introducció de WB en la dieta estava relacionat amb la seva mida de partícula. Els resultats obtinguts confirmaren que la inclusió de WB reduïa la població de *E. coli* en la digesta ileal, i encara més interessant, també reduïa l'adhesió del *E. coli* K88 a la mucosa ileal. Al mateix temps, el WB amb mida de partícula grollera reduí la diversitat de la microbiota intestinal en comparació amb el WB molturat.

La tercera prova (Prova 3, Molist et al., 2010a) fou dissenyada per esbrinar si els efectes positius del WB sobre la microbiota intestinal es devien a un efecte del WB sobre el trànsit intestinal dels animals. La hipòtesi del treball fou que la incorporació de WB en la dieta podia estimular el trànsit intestinal i reduir la paràlisis de la digesta intestinal dels garrins, causada per l'anorèxia que pateixen els animals en el període post-deslletament. En aquest experiment, el WB fou comparat amb un fàrmac que s'utilitza en medicina humana per tractar la diarrea que al mateix temps redueix el trànsit intestinal (loperamida). Els resultats de nou mostraren els efectes del WB sobre les característiques físico-químiques de la digesta (increment de la WRC) i la promoció de la fermentació intestinal (incrementant la concentració d'àcid butíric i disminuint la concentració dels isoàcids en la digesta intestinal). De forma inesperada, la loperamida incrementà el consum d'aliment i el creixement dels animals. Suggerírem que aquest efecte estava relacionat amb l'efecte analgèsic i l'activitat opioide d'aquest fàrmac en el tracte intestinal. No poguérem confirmar si el WB reduí el temps de trànsit intestinal o el possible rol que juga la modificació del temps de trànsit intestinal sobre els canvis de la microbiota intestinal.

En l'última prova (Prova 4, Molist et al., 2010b) la intenció era confirmar tots els resultats previs (reducció de la població d'enterobactèries i increment de la concentració d'àcid butíric) en un comparació entre la incorporació de WB amb la inclusió d'òxid de zinc (ZnO) en la dieta. El ZnO és un ingredient àmpliament utilitzat

en les dietes post-deslletament pel seu efecte antimicrobià similar al que s'obtenia amb la incorporació d'antibiòtics promotors de creixement (AGP) en el pinso, i per tant oposat a l'efecte promogut per la incorporació de fibra en la dieta. A més a més, considerant els resultats observats sobre la reducció de l'adhesió del *E. coli* K88 a la mucosa ileal promogut per l'addició de WB, es volia clarificar si el WB també podia exercir un efecte físic i blocar l'adhesió del *E. coli* K88 a la mucosa. Els resultats foren una mica sorprenents perquè s'observà una interacció negativa entre el WB i el ZnO sobre la microbiota intestinal. Aquesta interacció negativa s'associà a la presència de fitats en la dieta. Aquests resultats posaren de relleu la recomanació d'incorporar enzims (fitases) en les dietes després del deslletament amb l'objectiu d'incrementar la biodisponibilitat del zinc de la dieta. També detectarem una alta habilitat de la fracció soluble extreta del WB d'unir-se al *E. coli* K88 *in-vitro*. Aquest resultat ens permet suggerir que part dels efectes positius sobre la microbiota intestinal observats amb la incorporació de WB en la dieta eren deguts entre altres factors, a la seva capacitat de blocar l'adhesió de *E. coli* patògens a la mucosa intestinal.

Els resultats exposats en la present tesis, avalen l'estratègia d'incloure un nivell moderat de fibra (>60 g FND/kg per porcs entre 6 – 12 kg) en les dietes post-deslletament. Els resultats obtinguts mostren els efectes positius derivats de la inclusió d'una font de fibra insoluble, com WB, en la modificació de l'ambient intestinal i la instauració d'una microbiota saludable. Aquests efectes beneficiosos observats amb l'addicció de WB s'associaren a modificacions en les característiques físico-químiques de la digesta (increment de la WRC de la digesta) i amb la seva habilitat per blocar l'adhesió del *E. coli* a la mucosa ileal. Tot i així, el contingut en fitats d'aquest ingredient pot reduir la biodisponibilitat i l'eficàcia del ZnO en la dieta, fins i tot quan es subministra a dosis terapèutiques. És per aquest motiu que proposem considerar l'addició de fitases en dietes post-deslletament a base de cereals per: 1.- incrementar la biodisponibilitat de Zn, 2.- mantenir els efectes beneficiosos relacionat amb la inclusió de ZnO o WB en la dieta, o 3.- reduir la dosis de ZnO en la dieta.

SUMMARY

The objective of this thesis was to study whether the incorporation of fibrous ingredients in the diet of piglets would minimize the intestinal disorders that usually occur during the early period after weaning and facilitate the adaptation of the digestive system of the animals in the subsequent growing periods.
To achieve this goal, four trials (chapters 4 to 7) were designed.

In Trial 1 (Molist et al., 2009a), we first wanted to confirm some preliminary positive results associated with a higher growth rate of the animals obtained when an insoluble fibre source (wheat bran, WB) was introduced in post-weaning diets. At the same time, we wanted to assess whether this type of fibre source was appropiate for this period, or whether it would be more advantageous to incorporate a soluble fibre source (such as the sugar beet pulp, SBP). The study aimed to explore the effects of including two fibre sources (WB, insoluble and SBP, soluble) on the performance, the physicochemical properties of digesta and the metabolic activity and composition of the intestinal microbiota. Results showed that intestinal fermentation was low during the first week after weaning. The addition of WB or WB plus SBP in the diet increased intestinal fermentation and the concentration of butyric acid in the caecum digesta, and reduced the enterobacteria population in faeces. It was concluded that consumption of an insoluble fibre source during the first days after weaning (either WB or WB-SBP) modifies the physicochemical properties of digesta and affects the microbial colonization in the hindgut. We also speculated that the effects observed with the inclusion of WB could be associated with: 1.- changes in the physicochemical properties of digesta, such as the higher water retention capacity (WRC) and fermentation promoted in digesta, 2.- a physical effect related to its larger particle size or 3.- a reduction in the transit time of digesta.

In Trial 2 (Molist et al., 2009b), we wanted to confirm the referred reduction of the enterobacteria population promoted by WB, and its likely ability to reduce digestive disturbances after an experimental infection with *E. coli* K88. In addition, we wanted to clarify whether this effect of WB was related to its particle size. The results confirmed that WB inclusion reduced the *E. coli* population in the ileum

digesta and, more interesting, also reduced the *E. coli* K88 attachment to the ileum mucosa. Coarse particle size reduced the microbial diversity compared to finely milled WB.

The third trial (Trial 3, Molist et al., 2010a) was designed to elucidate whether the positive effects of WB on the intestinal microbiota could be due to an effect of WB on the intestinal transit of the animals. Our hypothesis was that incorporation of WB in the diet could stimulate the intestinal transit and so reduce the intestinal stasis of digesta in the piglets provoked by post-weaning anorexia. In this experiment, WB was compared with a drug used in human medicine to treat diarrhoea that slows the intestinal transit (loperamide). The results again showed the effects of WB on the physicochemical properties of digesta (increasing WRC) and the enhancement of gut fermentation (increasing butyric acid and lowering isoacid concentration associated to gut fermentation). Unexpectedly, loperamide increased the feed intake and animal growth. We suggested that this effect could be associated to its analgesic effect on and opioid activity in the intestinal tract. We were not able to confirm if WB reduced the intestinal transit time or the likely role of the modification of the intestinal transit time in the changes in intestinal microbiota.

The last trial (Trial 4, Molist et al., 2010b) intended to confirm all the previous results (the reduction of enterobacteria population and increasing the butyrate concentration) in a comparison between the incorporation of WB with the inclusion of zinc oxide (ZnO) in the diet. ZnO is a widely used ingredient in post-weaning diets producing antimicrobial effects resembling those of the antibiotic growth promoters (AGP) and therefore opposed to the inclusion of fibre in the diet. In addition, and considering the previous observed effects on the *E. coli* K88 adhesion to the ileum mucosa, we wanted to clarify whether WB could have a physical role on the blockage of the adhesion of *E. coli* K88 to the mucosa. The results were quite surprising because a negative interaction between WB and ZnO was observed on the intestinal microbiota, which was associated to the presence of phytates in the diet. These results highlighted the recommendation of incorporating enzymes (phytases) in the post-weaning diets in order to increase the bioavailability

of zinc. We also detected a high ability of soluble WB extract to bind *E. coli* K88 *in-vitro,* which suggests that part of the positive effects on the intestinal microbiota reported with the WB inclusion were due to its ability to block the adhesion of pathogenic *E. coli* to the intestinal mucosa.

Results exposed in this thesis, support the strategy of including a moderate amount of fibre (>60 g NDF/kg for pigs between 6 – 12 kg) in the diets of early weaned pigs. Our results show the positive effects of including an insoluble source, such as WB on the modification of the intestinal environment and the instauration of a healthy microbiota. These beneficial effects of WB inclusion were associated to changes on the physicochemical properties of digesta (like an increasing WRC of the digesta) and with its ability to block *E. coli* attachment to the ileum mucosa. However, the presence of phytates in this ingredient may also reduce the availability and efficacy of ZnO in the diet, even when it is provided at therapeutic doses. We propose the consideration of the inclusion of phytase in the post-weaning cereal based diets in order to: 1.- increase Zn biovailability, 2.- maintain the beneficial effects related to ZnO or WB inclusion, or 3.- reduce the therapeutic doses of ZnO in the diet.

INDEX

Chapter 1. General introduction p. 1

Chapter 2. Literature review p. 5
2.1. The weaning period in pigs p. 7
2.1.1. Acute phase of the post-weaning period p. 7
a) Stress p. 7
b) Post-weaning anorexia p. 8
c) Introduction of the solid diet p. 9
2.1.2. Progressive and mature phase p. 11
2.1.3. The characteristic microbiota during the weaning period p. 11
a) Colonization and establishment p. 11
b) Alterations of the microbiota: the appearance of diarrhoea p. 14
2.2. Post-weaning diet p. 16
2.2.1. Current post-weaning feeding strategies p. 17
a) Feeding of a highly digestible diet p. 17
b) Feeding of fibrous diets p. 19
2.2.2. Dietary fibre as an ingredient used in animal nutrition p. 20
a) Non-starch polysaccharides p. 22
b) Resistant starch p. 23
2.3. Host-dietary fibre-microbiota interactions p. 24
2.3.1. Interactions on the host's intestinal barrier p. 24
2.3.2. Modification of physicochemical properties and digesta transit time p. 26
2.3.3 Carbohydrates as anti-adhesive agents for infectious diseases p. 28
2.3.4 Modification of the intestinal microbiota p. 31
2.3.5 Carbohydrate fermentation p. 32

Chapter 3. Objectives and Experimental Design p. 37

Chapter 4. Trial I. Effects of the insoluble and soluble dietary fibre on the physicochemical properties of digesta and the microbial activity in early weaned piglets p. 41
4.1. Introduction p. 44
4.2. Materials and methods p. 44

4.2.1. Animals and diets p. 44
4.2.2. Experimental procedures and sampling p. 45
4.2.3. Analytical procedures p. 47
4.2.4. Statistical analyses p. 47
4.3. Results and discussion p. 47
4.4. Conclusion p. 52
4.5. Acknowledgments p. 53
4.6. References p. 53

Chapter 5. Trial II: Effect of wheat bran on the health and performance of weaned pigs challenged with *Escherichia coli* K88 p. 57
5.1. Introduction p. 60
5.2. Materials and methods p. 60
5.2.1. Animals and diets p. 60
5.2.2. Experimental procedures and sampling p. 61
5.2.3. Analytical procedures p. 61
5.2.4. Statistical analyses p. 62
5.3. Results and Discussion p. 62
5.3.1. Piglet performance p. 62
5.3.2. Faecal score and microbiological analysis p. 62
5.4. Conclusions p. 65
5.5. References p. 66

Chapter 6. Trial III. Administration of loperamide and addition of wheat bran to the diets of weaner pigs decrease the incidence of diarrhoea and enhance their gut maturation p. 69
6.1. Introduction p. 72
6.2. Material and methods p. 73
6.2.1. Animals and housing p. 73
6.2.2. Experimental procedures and sampling p. 75
6.2.3. Analytical procedures p. 75
6.2.4. Statistical analyses p. 76
6.3. Results p. 77
6.3.1. Experiment 1 p. 77
6.3.1.1. Animal performance, health status and nutrient digestibility p. 77

6.3.1.2. Fermentation end-products and quantitative changes in the microbial population of faeces p. 78
6.3.2. Experiment 2 p. 80
6.3.2.1. Digestion and morphometry of the intestinal mucosa p. 80
6.3.2.2. Physicochemical characteristics, fermentation parameters and microbial population of the colonic digesta p. 80
6.4. Discussion p. 81
6.4.1. The influence of wheat bran on the adaptation of piglets after weaning p. 81
6.4.2. The influence of loperamide on the adaptation of piglets to the diet p. 83
6.5. Conclusions p. 85
6.6. Acknowledgments p. 85
6.7. References p. 85

Chapter 7. Trial IV. The interaction between wheat bran and pharmacological doses of zinc oxide may reduce their effects on the intestinal microbiota of early weaned piglets p. 91
7.1. Introduction p. 94
7.2. Material and methods p. 96
7.2.1. Experiment 1: *In-vitro* adhesion test p. 96
7.2.1.1. Fibrous ingredients p. 96
7.2.1.2. Bacterial strains p. 96
7.2.1.3. *In-vitro* adhesion test p. 96
7.2.2. Experiment 2: *In-vivo* experiment p. 97
7.2.2.1. Animals and diets p. 97
7.2.2.2. Experimental procedures and sampling p. 99
7.2.2.3. Analytical procedures p. 99
7.2.3. Experiment 3: *In-vitro* wheat bran and zinc oxide interaction test p.100
7.2.3.1. Sample preparation p.100
7.2.3.2. Bacterial strains p.100
7.2.3.3. *In-vitro* test p.101
7.2.4. Statistical analyses p.101
7.3. Results p.101
7.3.1. Experiment 1: *In-vitro* adhesion test p.101

7.3.2. Experiment 2: *In-vivo* experiment p.102
7.3.2.1. Animal performance and health status p.102
7.3.2.2. Metabolic activity and composition of faecal microbiota p.103
7.3.3. Experiment 3: The *in-vitro* analysis of interaction between wheat bran and zinc oxide p.106
7.4. Discussion p.107
7.4.1. Potential of different fibrous substrates to bind *E. coli* p.107
7.4.2. The influence of wheat bran and zinc oxide on the adaptation of piglets after weaning p.108
7.4.3. Possible mechanism involved in the interaction between wheat bran and zinc oxide p.110
7.5. Conclusion p.111
7.6. Acknowledgments p.111
7.7. References p.111

Chapter 8. General discussion p.119
8.1. The likely role of fibrous ingredients in the post-weaning diet p.121
8.2. Effect of wheat bran on the pig performance and nutrient digestibility p.123
8.3. Effect of wheat bran on the composition of the intestinal microbiota p.124
8.3.1. Effect of wheat bran particle size p.124
8.3.2. Anti-adhesion activity of wheat bran p.125
8.3.3. Effect of wheat bran on the modification of the intestinal environment p.125
8.4. Negative interaction between wheat bran and zinc oxide p.126

Chapter 9. Conclusions p.129

Chapter 10. References p.133

Figures index

Chapter 2

Fig. 2.1. Review diagram of the acute phase of the post-weaning period p. 8

Fig. 2.2. Total bacteria counts (log CFU/ g FM) and percentage (% total bacteria) of coliforms, *Bacteroides* spp. and *Clostridium* spp. in piglet faeces from birth to 120 days of age (Castillo, 2006) p. 12

Fig. 2.3. Structure of the wheat kernel p. 23

Fig. 2.4. Diagram of the host intestinal protection mechanisms p. 25

Fig. 2.5. Schematic representation of the pathways for polysaccharides fermentation in the pig intestines (Bindelle et al., 2008) p. 35

Chapter 4

Fig. 4.1. Unbound water (A) and WRC (B) of colonic digesta on piglets fed experimental diets p. 48

Fig. 4.2. Total SCFA concentration in colonic digesta in early weaned piglets p. 49

Chapter 6

Fig. 6.1. Water retention capacity and unbound water of colonic digesta on piglets fed experimental diets (Experiment 2) p. 81

Chapter 7

Fig. 7.1. Dendogram illustrating the correlation between experimental diets: 4% wheat bran diet (WB) and 0.3% zinc oxide diet (ZnO), in t-RFLP banding patterns of faeces of post-weaning piglets (Experiment 2: *In-vivo* experiment). p.105

Tables index

Chapter 2

Table 2.1. Chemical analysis (%, as fed) of sow's milk and post-weaning diets p. 10
Table 2.2. Fimbria and enterotoxins associated with enterotoxigenic *E. coli* in piglets p. 15
Table 2.3. Fimbrial adhesins of enterotoxigenic *E. coli* and their receptors p. 16
Table 2.4. Composition of two different experimental diets (as fed, g/Kg) p. 18
Table 2.5. Classification according to the degree of polymerization, of the most common CH in plant material used in animal nutrition (Anguita, 2006) p. 21
Table 2.6. Fimbrial adhesins of enterotoxigenic *E. coli* and their adhesion carbohydrate factors p. 29
Table 2.7. Example of *in-vitro* inhibition test against enterotoxigenic *E. coli* in piglets p. 30

Chapter 4

Table 4.1. Composition and chemical analysis of pre-stater diets (g/kg dry matter) p. 46
Table 4.2. Average daily feed intake (ADFI), average daily gain (ADG), coefficient of total tract apparent organic matter (OMd) and starch digestibility (STd) in early weaned pigs p. 50
Table 4.3. Concentration (µmol/ g FM) of short-chain fatty acid (SCFA) and lactic acid on colon digesta and bacterial populations (enterobacteria and lactobacilli) measured by real-time PCR (log 16S rDNA gene copies/ g FM) on caecum digesta of piglet 15 days after weaning p. 51

Chapter 5

Table 5.1. Effect of wheat bran on the *E. coli* population in the feces (Log_{10} CFU/g digesta) and in the ileal mucosa (Log_{10} CFU/g of tissue) and *E. coli* K88 serotype counts in the ileum (Log_{10} CFU/g of tissue) and the faecal score in early weaned pigs p. 63
Table 5.2. Effect of wheat bran on the faecal and ileal digesta short chain fatty acids (SCFA) concentration in early weaned pigs challenged with ETEC p. 64

Table 5.3. Richness and diversity indices calculated from terminal restriction fragment length polymorphism data of the ileal digesta (collected on d 16 post-weaning) of nursery pigs challenged with ETEC p. 65

Chapter 6

Table 6.1. Diet composition and chemical analysis p. 74

Table 6.2. Body weight (BW), average daily feed intake (ADFI), average daily gain (ADG) and gain : feed ratio (G:F) in early weaned pigs (Experiment 1) p. 77

Table 6.3. Mortality, pigs with diarrhoea per treatment and coefficient of total tract apparent organic matter and crude protein digestibility in early weaned pigs (Experiment 1) p. 78

Table 6.4. Concentration of SCFA and bacterial population (enterobacteria and lactobacilli) on faeces of piglets, 13 d after weaning (Experiment 1) p. 79

Chapter 7

Table 7.1. Composition and chemical analysis of pre-starter diets (g/kg dry matter) (Experiment 2: *In-vivo* experiment) p. 98

Table 7.2. Detection times of bacterial growth $t_{OD=0.05}$ (h) for *E. coli* K88, non-fimbriated *E. coli* as a measure for adhesion in different fibre ingredients (Experiment 1: *In-vitro* adhesion test) p.102

Table 7.3. Body weight (BW), average daily feed intake (ADFI), average daily gain (ADG) and diarrhoea incidence in early weaned pigs (Experiment 2: *In*-vivo experiment) p.103

Table 7.4. Total and profile of short-chain fatty acids (micromole/ g FM) in day 12 after weaning, enteroccoci and *E. coli* counts (log CFU/ g FM) and lactobacilli population (log copies gene 16S rDNA/ g FM) in the faeces of piglets early after weaning (Experiment 2: *In-vivo* experiment) p.104

Table 7.5. Detection times of bacterial growth $t_{OD=0.05}$ (h) for *E. coli* K88, non-fimbriated *E. coli* as a measure of the ability of the *E. coli* strains to grow on different substrates (Experiment 3: *In-vitro* wheat bran and zinc oxide test) p.106

ABBREVIATIONS USED

ADF: acid detergent fibre
ADG: average daily gain
ADFI: average daily feed intake
AGP: antibiotic growth promoter
AMP: adenosyl monophosphate
Ara: arabinose
AST: apartate aminotransferase
BW: body weight
CFU: colony-forming unit
CH: carbohydrates
CP: crude protein
CT: control diet
DE: digestible energy
DF: dietary fibre
DGGE:denaturing gradient gel electrophoresis
DM: dry matter
dp: degree of polymerization
E. coli: *Escherichia coli*
EGF: Epidermal growth factor
ETEC: enterotoxigenic *E. coli*
FI: feed intake
FM: fresh matter
Fru: fructose
FS: faecal score
FucOS: fucosylated oligosaccharides
Gal: galactose
Gal: galactose
GIT: gastrointestinal tract
Glu: glucose
Hb: haemoglobin
ICE: incidence-based coverage estimator
Ig: immunoglobulin
IL: interleukin
iNSP: insoluble non-starch polyaccharides
Lys: lysine
LOP: loperamide
LT: heat labile toxin
Man: manose
MM mean: Michaelis-Menten mean
MOS: mannan-oligosaccharides
MTT: minimum transit time
NC: negative control diet
NDF: neutral detergent fibre
Neu5Ac: N-acetylneuraminic acid
NeuGc: N-glycolylneuraminic acid
NRC: National Research Council
NSP: non-starch polysaccharides
OM: organic matter
OMd: organic matter digestibility
PA: phytic acid
PC: positive control diet
PWC: post-weaning collibacilosis
RBC: red blood cells
RS: resistant starch
SBP: sugar beet pulp
SCFA: short chain fatty acids
sNSP: soluble NSP
STa: heat stable toxin A
STa: heat stable toxin B
TRF: terminal restriction fragments
t-RFLP:terminal restriction length polymorphism
UPGMA: un-weighted pair-group method with averaging algorithm
VFA: volatile fatty acids
WB: wheat bran/ wheat bran diet
WBc: wheat bran coarse diet
WBC: white blood cells
WBf: wheat bran finely milled diet
WB-SBP: WB and sugar beet pulp diet

WM: wheat middlings diet
WRC: water retention capacity
Xyl: xylose
ZnO: zinc oxide

CHAPTER 1

General Introduction

One of the main restrictions in the pig industry is the survival and growth of piglets in the post-weaning period. The accumulation of different stress factors (environmental, social and dietary), together with the immaturity of their intestinal and immune systems, may lead the animals to develop anorexia, intestinal stasis, a low rate of feed digestion and the risk of diarrhoea (Lallès et al., 2007). The intensity of this crisis may increase mortality and reduce growth in animals from wean to finish. The most common strategy to overcome these problems and successfully pass through this phase was the introduction of AGP in the feed. However, concerns regarding cross-resistance of pathogens in humans have resulted in a total ban of antibiotics as growth promoters in livestock in the European Union. Alternatively, ZnO is being incorporated at therapeutic doses, with antimicrobial results resembling those of antibiotics. However, the environmental impact of high levels of Zn excretion makes this practice questionable. Therefore, there is a need to seek nutritional strategies alternatives to AGP and ZnO.

One strategy is the incorporation of dietary ingredients that could allow the establishment of a beneficial flora in the gastrointestinal tract (GIT) to prevent the proliferation of pathogenic bacteria and at the same time prepare their digestive tract for the growing period. In this respect, there is a consensus about the effects of some alternatives substances to AGP that can modify the intestinal microbiota such as: organic acids, probiotics, prebiotics or plant extracts (Partanen and Mroz, 1999; Zimmerman et al., 2001; Manzanilla et al., 2004). On the other hand, there remains some controversy about the inclusion of fibrous ingredients in post-weaning diets. Dietary fibre (DF) includes lignin, non-starch polysaccharides (NSP) and starch that are resistant to digestion in the small intestine, and which ferment to some extent in the hindgut (Trowell et al., 1976). Therefore, this element includes a wide variety of ingredients, and therefore the fermentation patterns and the physicochemical properties may vary from one to another.

Traditionally, fibre has been considered practically an antinutritional factor for piglets because it may reduce feed intake and nutrient digestibility. Authors against the inclusion of fibre ingredients in the post-weaning diet also argue that changes to the physicochemical characteristics of digesta with fibre may enhance the proliferation of pathogenic bacteria and the emergence of post-weaning diarrhoea. In this regard,

McDonald et al. (1999) reported that the inclusion of soluble and viscous fibre in the diet increased the viscosity of the intestinal digesta favouring the proliferation of pathogenic bacteria. On the other hand, authors that support the inclusion of fibre ingredients in the diet report beneficial effects of the inclusion of soluble but non-viscous fibre sources (Bikker et al., 2006; Wellock et al., 2007) or insoluble fibre sources (Freire et al., 2000; Mateos et al., 2006) attributed to the reduction of the protein fermentation (Hermes et al., 2009) and due to changes in the environment of the GIT. However, among these authors there is no consensus on the type of fibre to be included in the diet nor on the level and the duration of any such inclusion. In the same way, there are few studies that explain the interaction between dietary fibre (DF) type and the animal intestinal microbiota (composition or metabolic activity) as well as the interaction of fibre with other ingredients commonly used in post-weaning diets such as ZnO that is used worldwide as the main alternative to AGP.

It therefore seems opportune to conduct a comprehensive study about the introduction of fibre in the post-weaning diet and its effect on the animal performance, their gastrointestinal system and on intestinal microbiota, taking into account the interaction with other ingredients of the diet. The final objective will be to give a dietetic recommendation to nutritionists in the swine industry.

CHAPTER 2

Literature Review

2.1. The weaning period in pigs

Weaning is defined as that period during which piglets suffer a forced separation from the sow. In commercial conditions, this phase happens in an abrupt and premature way at 21 to 28 days old; in contrast to wild animals in which piglets stop suckling at an approximate age of 10 weeks. After weaning, piglets are mixed with others from different dams in a new space and environment with a new diet, where they pass from a highly digestible milk diet to less digestible solid diet. This situation causes stress and result in a transitory period of anorexia (McCracken et al. 1999) (Fig. 2.1). The stress and reduced food consumption lead to intestinal inflammation which affect the microbal balance, and the enzymatic and immunity activity of the small intestine, increasing the risk of diarrhoea (Pluske et al., 1997; Lallès et al., 2004).

From a physiological point of view this process can be divided into two periods (Montagne et al., 2007).

2.1.1. Acute phase of the post-weaning period

The first period, which is considered to last 5 days after weaning, is characterized by a deterioration of gastrointestinal integrity. Although the effects begin in the stomach with a decrease in rate of gastric emptying (Liesnewska et al., 2000), it is the intestinal activity that is most altered (Lallès et al., 2007). It is possible to find a reduction of the villus height and the enzymatic activities of some carbohydrases such as lactasa and sucrase (Pluske et al., 1995). The former, will decrease its levels throughout the post-weaning period, and sucrase will recover the basal levels in the second phase. Changes in the intestinal structure can be explained by:

a) Stress

There is no agreement between authors in the way that stress affects the intestinal architecture. Pluske and Williams (1996a) reported that changes in the intestinal activity and structure observed in the post-weaning period could be caused by the stress that the animals suffer or due to the low feed intake. However, the separation of piglets from the sows, together with the mixing with other animals and

the need to establish hierarchies in the group are situations that are stressful enough to promote physiological changes in the animals (Pluske et al., 1997). In the rat, repeated separation from the mother for 3 h has been shown to have potentially deleterious effects. Separated rat pups showed reduced hippocampal glucocorticoid receptors, elevated basal plasma glucocorticoids (Plotsky and Meaney, 1993) and became hypperresponsive to stressors during behavioural development (Ladd et al., 2000). In weaning pigs of below 28 days, the stress is accompanied by increases in vocalisation (Mason et al., 2003), increased anxiety (Dantzer and Mormede, 1983) and an increase in the basal cortisol levels (Worsaae and Schmidt, 1980).

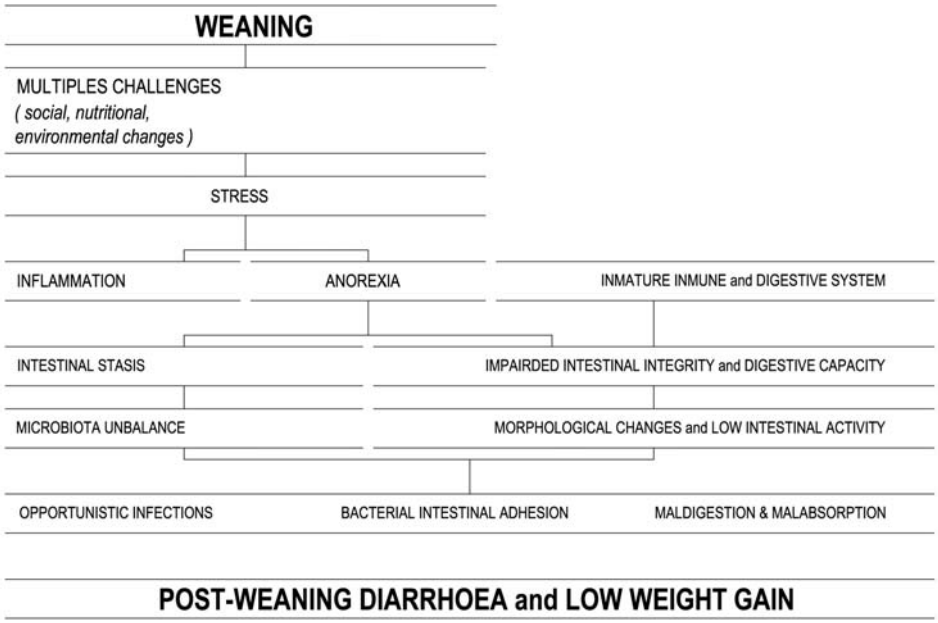


Fig. 2.1. Review diagram of the acute phase of the post-weaning period.

b) Post-weaning anorexia

Another common situation in the first phase of the post-weaning period is anorexia. Although 50% of weaned piglets consume their first meal within 24 h after weaning, 10% of pigs do not eat until ± 48 h after (Brooks et al., 2001). Thus, energy requirements for maintenance are only met 3 days after weaning, and it can

take 8 – 14 days for piglets to recover their pre-weaning level of energy intake (Le Dividich and Sève, 2002). The low feed intake causes a reduction on the input of nutrients to the intestinal mucosa, mainly in the proximal part of the small intestine where the principal source of energy comes from the nutrients of the diet. The ileum and the intestinal crypts take and receive energy from the arterial blood, so they will be less affected by the process of anorexia. Another consequence of anorexia is a lower pancreatic enzyme secretion and a decreseased intestinal integrity. Boudry et al. (2004) reported transient increases in the net ion transport in the ileum and colon and in glucose absorption capacity in the jejunum and decreased jejunal electric resistance in piglets which had fasted for 2 days after weaning.

c) Introduction of the solid diet

Weaning implies the separation of piglets from their mothers, and consequently an end to the consumption of the sows' milk. Colostrums and milk are rich in growth factors and bioactive compounds that are necessary for the differentiation and development of the small intestine. The bioactive compounds involved in the small intestinal development in young pigs include epidermal growth factor (EGF), polyamines, insulin, the insulin-like growth factors (IGF), immunoglobulin's, bioactive peptides or nucleotides (Simmen et al., 1990; Odle et al., 1996; Kelly et al., 1991). At weaning, the input of these substances stops drastically leaving the intestinal epithelium orphan of these nutrients. Petrovic et al. (2009) found higher indices for RBC, Hb, WBC, total Ig, AST, urea and Se; and lower indices of albumin, pancreatic amylase, glucose, Ca, vitamin A and vitamin E in the blood of post-weaning animals compared to the end of suckling period, indicating that dietary changes during suckling and post-weaning periods affected the majority of blood indices in piglets. Along the same lines, Burrin et al. (1995) reported that pigs deprived of colostrums but which received milk replacement fortified with IGF-1 for four days had greater intestinal weight and higher villi in the jejunum than their counterparts. Martínez-Puig et al. (2007) also demonstrated the positive effects of including nucleotides in the post-weaning diet. Dietary supplementation with 1000 ppm of a yeast extract containing nucleotides in the

range of sow's milk at lactation improved the growth performance of early weaned piglets.

Moreover, weaning is also a change to a solid diet containing a high level of vegetable ingredients and usually a higher dry matter (DM) content (Table 2.1). The solid diet is less palatable than a liquid diet (Deprez et al., 1987), which results in a lower feed intake and slower growth of the piglets (Pluske et al., 1996b,c). In addition to this the transient hipersensitivity of the post-weaning piglets towards some compounds in the new diet is remarkable. It has been demonstrated (Hampson, 1987) that animals which receive creep feed develop immunological tolerance towards the post-weaning diet; diminishing the risk of post-weaning diarrhoea compared to animals that had not consumed solid feed during lactation.

Feed composition affects palatability, but also has physiological implications in the digestive tract. A post-weaning diet, rich in fibre ingredients will promote development of the hindgut and a raise the fermentative activity of the intestine. Fermentation will reduce pH and will increase the level of short chain fatty acids (SCFA) compared to suckling piglets (Castillo et al., 2007a). However, in the first days after weaning, it has been demonstrated that the sort of fibre and its concentration in the diet will not affect the SCFA produced derivatives of hindgut activity (Laerke et al., 2007).

Table 2.1. Chemical analysis (%, as fed) of sow's milk and post-weaning diets.

References	Dry matter	Crude protein	Fat	Lactose
Sow's milk				
Jackson et al., 1995	18.3	5.5	7.0	4.6
Garst et al., 1999	19.0	5.9	7.2	4.7
Hurley et al., 2000	20.1	5.6	8.3	5.0
Post-weaning diets				
O'Connell et al., 2005	89.9	20.5	7.5	17.0
Pierce et al., 2005	91.7	21.9	7.5	17.5
Pierce et al., 2007	90.8	16.2	7.1	21.4

2.1.2. Progressive and mature phase

This phase covers the period between 5 and 15 days post-weaning. It is characterized by a progressive recuperation of some parameters that indicate the adaptation of the piglet to its new diet. These parameters could be the increase of the mucosa mass in the jejunum due to the growth of the intestinal villus related to the arrival of nutrients in the intestinal lumen; and the increase of the pancreatic mass, which shows the recuperation of the enzymatic activity. Nevertheless, the defining parameters of animal maturation in this phase are: maltasic activity, glucose absorption, the presence of enteroccoci and lactobacilli in the colon digesta and the reduction of the pH in the caecum and colon (Montagne et al., 2007). Other authors have reported other indicators for the new period of the animal's adaptation, such as the increase of the concentration of SCFA in the faeces due to the fermentation of carbohydrates (CH) in the hindgut, a higher propionic or butyric acid ratio and a lower acetic acid concentration, due to the increase of the microbial and diversity population in the intestine (MacFarlane and McBain, 1999). Another effect observed is the increased DM content in the faeces, which is associated with a higher SCFA concentration and water absorption from the lumen of the GIT (Awati et al., 2006).

2.1.3. The characteristic microbiota during the weaning period

The instauration of a stable microbiota in the GIT in the post-weaning pig is a keypoint for an optimum health of the animals, which will determine the gains or losses in the following phases of the pig production. It is therefore, very important to understand the mechanisms involved in the establishment of a beneficial or pathogenic microbiota in the GIT of weaned pigs.

a) Colonization and establishment

The description of the different phases of the piglet's adaptation after weaning indicates a relevant role for the intestinal microbiota. The development and instauration of the intestinal microbiota is a complex process of natural selection similar to that of humans and the majority of the livestock animals (Mackie et al., 1999). It begins with a phase characterized by a fast colonization of the environmental bacteria followed by different stages where dominant groups of

microorganisms are established. This process continues with the growth of the animals and ends with giving each animal a dynamic and characteristic bacterial population (Zoetendal et al., 2001). The establishment of the GIT microbiota is determined by different mechanisms. Some of them are given by the host. In the stomach and the small intestine, low pH and bile secretion prevent the proliferation of many microorganisms, causing qualitative and quantitative differences in the microbial population along the GIT. Another factor is diet. Clear examples of the differences can be observed between the suckling and weaning periods. Weaned pigs had a lower lactobacilli population in the ileum digesta as well as a lower bifidobacteria and enterococci population (Jensen et al., 1998).

The colonization period is divided into three distinct consecutive phases. The first phase encompasses the first week of life of the animals; the second includes the whole suckling period and the third starts in the post-weaning period (Swords et al., 1993).

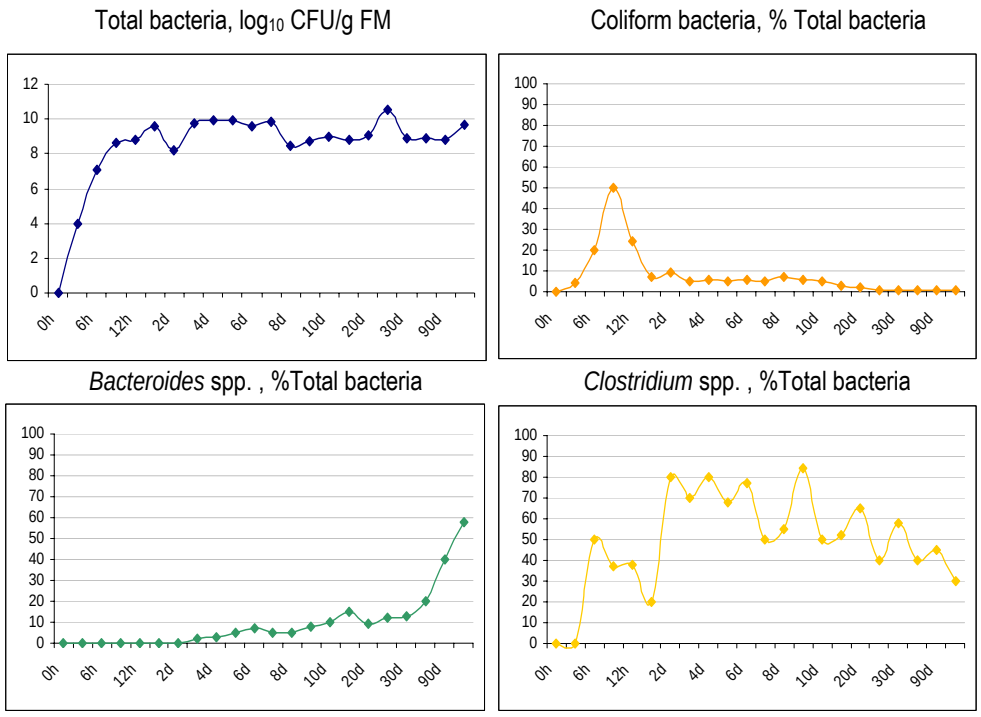


Fig. 2.2. Total bacteria counts (log CFU/g FM) and percentage (% total bacteria) of coliforms, *Bacteroides* spp. and *Clostridium* spp. in piglet faeces from birth to 120 days of age (Castillo, 2006).

The presence of bacteria in the uterus of the mother has been demonstrated (Jiménez et al., 2005). Immediately after birth, the microbial population of the GIT has to change from a simple to a complex community (Konstantinov et al., 2004). The complexity increases due to the environment and/or from the mother's anal and vaginal bacteria. These bacteria are transferred to the neonate by oral suckling. In particular, faeces from the sow are an important source of microorganisms for the GIT of piglets. Normally, the first colonizers are aerobic bacteria or anaerobic facultative bacteria such as lactic acid bacteria, enterobacteria and streptococci (Stewart, 1997). Therefore, 2 h after birth it is possible to find *Escherichia coli* (*E. coli*) and streptococci, which 5 or 6 h later will grow to 10^9 – 10^{10} CFU/ g faeces respectively (Ewing and Cole, 1994). The first bacteria colonizers are responsible for creating a favourable environment for the establishment of strict anaerobe microorganisms, such as *Bacteroides* spp*., Bifibobacterium* spp. and *Clostridium* spp*.,* after the first week of lactation. This pattern will be maintained while the animals consume milk (Hammes et al., 1991; Conway, 1994) (Fig. 2.2.).

At weaning, the intestinal microbial population becomes unstable and suffers a reduction in diversity. Diversity will increase again one week after weaning (Jensen et al., 1998). The introduction of solid feed obligate anaerobes to increase in number and diversity until an adult-type pattern is achieved (Konstantinov et al., 2004; Inoue et al., 2005). In this phase, it has been stated that the change from gram positive anaerobic bacteria to gram negative bacteria of *Bacteroides* genus is the cause of one of the major bacterial groups in the intestinal ecosystem of an adult pig (Inoue et al., 2005). In contrast to adults, the neonatal and weaning piglets are highly susceptible to enteric diseases (Hopwood and Hampson, 2003). In the immediate post-weaning period the balance between the development of commensal microbiota and the establishment of a bacterial intestinal disease can easily tip towards disease expression (Hopwood and Hampson, 2003). Traditionally, lactobacilli and enterobacteria population have been chosen as special microbial groups to determine the intestinal health of piglets. Therefore, the ratio between these two bacterial groups has been used routinely as an indicator (Castillo et al., 2007a). It is recommended that the lactobacilli population overcome the enterobacteria population indicating a higher resistance of the animals against the

intestinal pathogens. Castillo et al. (2007a) reported a negative ratio in the cecal digesta in post-weaning piglets compared to a control group of suckling animals, showing the negative effect of weaning. In the same work, the differences between these two microbial populations were exposed in a dendogram obtained by the terminal restriction fragment length polymorphism (t-RFLP) analysis of the local cecal digesta. Analysis proved two differentiated clusters between suckling and weaned piglets. Along the same lines, Su et al. (2008) also reported predominant bands in a denaturing gradient gel electrophoresis (DGGE) analysis in post-weaning piglets. *Lactobacillus spp.* bands disappeared and were replaced by pathogenic species, such as *Peptostreptococcus anaerobius, Moraxella cuniculi, Streptococcus suis* and *Porphyromonas catoniae*.

b) Alterations of the microbiota: the appearance of diarrhoea

In the pig industry, diarrhoea is probably the main disease in the post-weaning period (from 4 to 14 days post-weaning) and is responsible for significant economic losses. The post-weaning colibacillosis is produced by a limited range of enterotoxigenic serotypes of *E. coli* (Table 2.2) that cause hipersecretory diarrhoea due to the release of specific enterotoxins in the intestinal tract. Among them, they include a heat-labile toxin (LT) that binds to the enterocytes and releases some active subunits that can for example, activate irreversibly the adenylylate cyclase and increase cyclic adenosyl monophosphate (AMP) production leading to the secretion of: chloride ions, sodium, bicarbonate and water into the intestinal lumen (Fairbrother, 1992). It is very common to find haemolytic *E. coli* in the faeces of piglets during the first week after weaning; but the number is higher in diarrheic pigs (Hampson, 1987). The critical point of this proliferation is the colonization of the small intestine by the adhesion of the *E. coli* fimbria to the mucus and the intestinal receptors (glycoproteins) (Table 2.3). Colibacillosis in Spain is usually associated to the *E. coli* serotypes that produce F4 fimbria. The receptor for the F4 fimbria disappears at the end of the post-weaning period, so *E. coli* has a brief opportunity to adhere and proliferate (Conway et al., 1990).

Table 2.2. Fimbria and enterotoxins associated with enterotoxigenic *E .coli* in piglets.

Fimbria	Enterotoxins[a]	Host	References
K88ab, ac, ad (F4)	LT, Sta, STb	Suckling and weaned pigs	Guinee et al., 1977
K99 (F5)	STa, STb	Calves and piglets	Wilson and Francis, 1986
987P (F6)	STa, STb	Neonatal pigs	Moon et al., 1980
F18ac	LT, Sta, STb	Weaned pigs	Dean-Nystrom et al., 1993

[a]LT = heat-labile toxin, STa = heat stable toxin a, STb = heat stable toxin b.

It is well established that adherence of pathogenic bacteria to intestinal epithelium is a pre-requisite for colonization and infection of the GIT. The intestinal epithelium is not just a physical barrier that prevents unwanted bacteria from gaining access to essential organs; it also provides a surface covered by specialized cells producing mucus, antimicrobial peptides and antimicrobial molecules, which together with resident microbiota provide the front line of defence against pathogenic microorganisms. The GIT bacteria may be free-living or attached to mucus, mucosa surface, food particles or digestive residues. Complex CH structures (polysaccharides or glycans) usually found as glycoproteins, glycolipids, mucins and glycosaminglycans cover mucosal surfaces in the intestine and are potential adherence sites for intestinal bacteria (Table 2.3). Pathogens infect their target host tissues through a series of stages that begin with attachment to cell-surface glycan binding sites. The attached bacteria produce microcolonies, leading to the development of biofilms (Kleessen and Blaut, 2005).

The most common means of adhesion of numerous bacteria, are surface lectins that combine with complementary CH present on the host cell surfaces (Sharon and Lis, 1989). They serve as virulence factors of the organisms and are among the determinants of their organ and tissue tropism (Kyogasima et al., 1989). Most bacterial lectins are surface-bound and are known as fimbriae or pili. There is a high level of specificity between carbohydrate and the bacterial surface lectins. For example *E. coli* K99 binds to glycolipids containing N-glycolylneuraminic acid (NeuGc), in the form of NeuGc α2-3Galβ1-4Glcβ 1-1-cer, but not to those that contain N-acetylneuraminic acid (Neu5Ac). These two sugars differ in only a single

hydroxyl group, present in the acyl substituent in the 4-NH group of NeuGc acid and absent in that of Neu5Ac acid. The NeuGc acid is found on intestinal cells of newborn piglets, but it disappears when the animals develop and grow. This explains why *E. coli* K99 can cause diarrhoea in piglets, but not in adult pigs or humans.

Table 2.3. Fimbrial adhesins of enterotoxigenic *E. coli* and their receptors.

Fimbria	Lectin	Intestinal receptor molecule	References
K88ab	FaeG (ab)	b: Transferrin N-glycan (74 kDa)	Grange and Mouricout, 1996
		bc: IMPTGP (210 – 240 kDa)	Erickson et al., 1994
		bcd: Glycoproteins (45 -70 kDa)	Willemsen & de Graaf, 1992
K88ac	FaeG (ac)	bc: IMPTGP (210 – 240 kDa	Erickson et al., 1994
		bcd: Glycoproteins (45 -70 kDa)	Willemsen & de Graaf, 1992
K88ad	FaeG (ad)	d: Neutral glycosphingolipids	Grange et al., 1998
		bcd: Glycoproteins (45 -70 kDa)	Willemsen and de Graaf, 1992
K99	FanC	N-Glycolylsialoparagloboside	Kyogashima et al., 1989
		N-glycolyl - GM3	Kyogashima et al., 1989
987P	FasG	Sulfatide	Dean, 1990
		Proteins (32 – 35 kDa)	Khan and Schifferli, 1994
	Fas A	Ceramide monohexoside	Khan et al., 1996
F18ac	FedF	Unknown	Meijerink et al., 1997

2.2. Post-weaning diet

Diet is the link between the animal and its intestinal microbiota. The intestinal microbial population of healthy animals is subjected to modifications in terms of predominant species according to the diet. Therefore the post-weaning diet will affect the instauration of the microbiota in the GIT and the performance of the animals after weaning. As it was described earlier, weaning represents a big change in the feed composition for the piglet. The main differences in comparison with sow's milk are the lower content of water in the feed (the piglet must learn to drink water to quench its thirst), the incorporation of vegetal ingredients in the diet, and the lower

content of fat and lactose. As has been mentioned, diet composition or its presentation are factors influencing the growth and stability of microbial populations, including those causing diarrhoea. The strategy of prevention of post-weaning digestive diseases has generally involved the incorporation of antimicrobial compounds in feed, including antibiotics and ZnO. However, social pressure and legislation against their use is growing (due to bacterial resistance and the emergence of environmental problems), which turn the focus toward an optimization of the piglet's digestive processes and its natural mechanisms of defence. For example, the presence in the diet of some anti-nutritional factor in some ingredients like soya bean meal, rich in lectins, tannins and α-amylase inhibitors that can decrease production by affecting the gut structure and function (Lallès et al., 1993). In cereals, it is also necessary to consider the phytate content and the likely interaction with bivalent cations, such as Zn, which may affect their bioavailability and lead to an increased mineral excretion (Champagne and Fisher, 1990).

2.2.1. Current post-weaning feeding strategies

Nowadays, cereals are one of the major ingredients in the animal feed. The most common cereals in the ration of post-weaning piglets are maize, barley, wheat, oats and rice. The inclusion, presentation and treatment of the cereals of the diet may rise to different animal responses. There are different dietary strategies which aim to improve the adaptation of the animals during the post-weaning period (Table 2.4).

a) Feeding of a highly digestible diet

This first strategy consists of offering the young animals an extremely digestible and very costly post-weaning diet. These rations are based on palatable and digestible ingredients, such as milk by-products, rice or animal proteins. Less digestible ingredients, are included in the diet as the animals grow older. The aim of this strategy is to promote feed consumption by the piglets in order to reduce the problems associated with anorexia, and facilitate digestion to avoid the accumulation of indigestible substrate and the proliferation of pathogenic bacteria. The incorporation of rice in the diet has become popular as its introduction has been

associated with improvements in feed consumption due to its high palatability (Solà-Oriol, 2008) and with a reduction of post-weaning diarrhoea (Mateos et al., 2001). Furthermore, rice is more highly digestible, it has a lower content of non-starch polysaccharides (NSP) and the presence of antisecretory factors that may contribute to the reduction in the incidence of diarrhoea. McDonald et al. (1999) reported that a post-weaning diet based on cooked rice and animal protein protected piglets against post-weaning diarrhoea.

Table 2.4. Composition of two different experimental diets (as fed, g/Kg).

	High digestible diet [a]	High fibre diet [b]
Cooked rice	699.4	-
Corn starch	-	408.0
Wheat bran	-	200.0
Dry milk	79.4	100.0
Fish meal	151.7	100.0
Potato protein	-	100.0
Soya bean oil	-	50.0
Blood meal	25.3	-
Vitamin and mineral premix	21.5	25
Dicalcium phosphate	17.9	16.0
Meat and bone meal	3.6	-
Synthetic amino acids	1.0	1.0

[a] High digestible diet adapted from Mc Donald et al., (2001)
[b] High fibre diet adapted from Freire et al., (2000)

The same author (McDonald et al., 2001) demonstrated that adding highly viscous carboximetilcellulose to this diet increased diarrhoea due to the accumulation of indigestible feed, the increase in the viscosity of digesta, and the proliferation of pathogenic bacteria. In another study, McDonald et al. (2001) indicated that the inclusion of vegetable ingredients in a post-weaning diet diminished the ratio of the villus height and crypt's depth, aggravating the problem related to the low digestion. In contrast, the administration of a low fibre diet with highly digestible ingredients reduced the diarrhoea in post-weaning piglets (Montagne et al., 2003). By contrast, Kim et al. (2008) reported that the inclusion of

extruded rice determined a higher incidence of post-weaning diarrhoea compared to wheat based diet. The authors suggested that these differences between cooked and extruded rice were due to the level of resistant starch (RS). Cooked rice may have supplied more RS compared to the extruded rice. Authors suggest the important role of the RS to reduce the post-weaning diarrhoea. The RS may compensate for the lower fibre content of the rice.

b) Feeding fibrous diets

On the other hand, other researchers propose to control the intestinal disbiosis by promoting the establishment of a healthy microbiota in the GIT. The idea is to promote the growth of a more robust and stable microbiota. These diets are usually based on raw and whole grain cereals that cause a comparative increase the fibre concentration of the post-weaning diet. These diets are cheaper but the effects on pig production are less controlled than those used in the first strategy. A report that defends this thesis was done by Montagne et al. (2004). They showed that the substitution of animal protein for vegetable protein in post-weaning diets did not increase the incidence of *E. coli*. This result indicates that the instauration of healthy and stable microbial population in the digestive tract due to the fibre incorporation had positive effects on the animal health. The fibre content of the diet greatly influences the digestive process, the physicochemical properties of digesta (Canibe and Bach Knudsen, 2002), the morphology of the GIT (Jorgensen et al., 1996) and the maturation and integrity of the mucosa (Brunsgaard, 1998). The described effects of fibre on the enumerated parameters could be due to its physicochemical properties or indirectly determined by its fermentation or the ability to modify the GIT microbiota balance.

However, sometimes an increase in the digestive fermentation is not correlated with an improvement of animal performance due to a lower availability of energy for the animals. Le Goff and Noblet (2001) reported that for each gram of neutral detergent fibre (NDF) per kilogram of feed, fat digestibility decreased in 0.02 grams of digestible fat/ kg; and the energy also decreased 0.1% points for each gram of increasing of NDF/kg DM. Other authors reported a negative correlation between the carcass weight and the percentage of fibre in the diet (Pluske et al.,

2003). However, some authors suggested that animals may have minimum requirements of fibre to optimize their digestive function (Mateos et al., 2006; suggested >60 g NDF/kg for pigs between 6 – 12 kg). In piglets fed with a rice-based diet, the incorporation of oat hulls reduced the diarrhoea incidence without affecting animal performance (Mateos et al., 2006; Kim et al., 2008).

There are disagreements surrounding the source of fibre that should be incorporated in the post-weaning diet. In general, soluble fibre fractions are more fermentable and are likely to be more viscous; which may slow-down the digesta transit time during the first days after weaning. On the other hand, the insoluble fractions are less fermentable; they have higher WRC and a higher ability to reduce the digesta transit time. In this way, it seems important to consider the level of fibre and its composition in the diet. Hogberg and Lindberg (2006) evaluated the effect of the NSP level (95 and 203 g/kg) and its composition (normal or mainly insoluble based on oat hulls and WB) in semi-synthetic diets. The inclusion of low levels of NSP (109g/kg) mainly insoluble (87g/kg) increased the feed consumption and animal performance. Consistent with previous work done, a diet with a lower NSP and lower insoluble NSP (iNSP) content showed the higher digestibility indeces but lower levels of ingestion and weight gain. Higher levels of insoluble (173 g/kg) fibre (203 g/kg) made the organic matter more digestible but, promoted higher hindgut fermentation. Although it is difficult to make a precise recommendation, different works (Hogberg and Lindberg, 2006; Mateos et al., 2006) agree with the idea that starter diets should have a minimum level of fibre, of which most should be insoluble, to facilitate digestive function and digesta transit, to stimulate feed consumption and weight gain in the animals soon after weaning. Mateos et al. (2006) suggested that these minimum requirements could be around 60g NDF/kg or 109g NSP/kg and 87 g insoluble NSP/kg as described by Hogberg and Lindberg (2006) for piglets between 6 and 12 kg.

2.2.2. Dietary fibre as an ingredient used in animal nutrition

The two strategies presented above show the complexity of the CH composition in different ingredients. The term carbohydrate (Pigman and Horton, 1972) includes a large number of structures with different compositions and

complexity (Table 2.5). Another specific term that it is commonly used in animal nutrition studies is DF. The DF is defined as all plants polysaccharides and lignin that are resistant to hydrolysis by mammal digestive secretions (Trowell et al., 1976).

Table 2.5. Classification according to the degree of polymerization, of the most common CH in plant material used in animal nutrition (Anguita, 2006).

Group (dp)[a]	Subgroup	Monosaccharide[b]	Linkage
Sugars	Monosaccharides	Glu, Gal, Fru,	
(1-2)		Xyl, Ara, Man	
	Disaccharides	Glu, Fru	α,β-(1→2)
		Glu	α-(1→4)
		Glu, Gal	β-(1→4)
		Glu	β-(1→4)
Oligosaccharides	α-Galactosides	Gal, Glu, Fru	α-(1→6)/ (1→2)
(3-9)		Gal, Glu, Fru	α-(1→6)/ (1→2)
		Gal, Glu, Fru	α-(1→6)/ (1→2)
	Malto- oligosaccharides		
		Glu	Linear α-(1→4)
	Fructo- oligosaccharides		
		Glu, Fru	Linear β-(1→4)
Polysaccharides	Starch	Glu	Linear α-(1→4)
(>10)		Glu	Branched α-(1→4)/(1→6)
	NSP	Glu	Linear β-(1→4)
		Xyl, Ara	Linear β-(1→4)
		Glu	Mixed β-(1→3)/ (1→4)
		Galacturonic acid	Linear α-(1→4)

[a] (dp) degree of polymerization

[b] Glu, glucose; Gal, galactose; Fru, fructose; Xyl, xylose; Ara, arabinose; Man, manose.

DF covers a wide range of CH known as NSP that include pectins, cellulose, hemicelluloses, β-glucans and fructans. Oligosaccharides and RS are also considered in the DF fraction.

a) Non-starch polysaccharides

The NSP fraction is constituted by a varied group of polysaccharides (Table 2.5). Most of them belong to the associated vegetal cell wall or to the replacing proteins and phenolic compounds (Selvendran, 1984). There are no endogen enzymes secreted in the stomach, in the small intestine or in the epithelial surface of the intestinal villi that can hydrolyze the glycoside linkages of the NSP. These compounds can only be degraded by microbial enzymes. Due to the high transit time of digesta in the small intestine, the microbial fermentation of these compounds in that compartment is limited. Thanks to the anatomical (pigs have a small caecum but a large colon compared to other non-ruminants herbivores) and physiological (anti-peristaltic movements increase the digesta transit time in this organ) characteristics of the hindgut of pigs, the resident microbiota that inhabit this organ may recover part of their energy from undigested substrates.

Due to its internal structure, cellulose is the most highly insoluble compound in water and one of the diet components least digested by the young animal (Gardner and Blackwell, 1974). Pectins are formed by polymers of glucoronic and galacturonic acid and most of them are soluble in water. They have the properties of increasing WRC, viscosity, buffering capacity, enzymatic pre-caecum resistance and fermentability in the hindgut. They are only degraded by 10% in the small intestine. Their fermentation in the large intestine mainly produces acetate.

The concentration of the NSP in plants depends on the botanical species and the part of the plant under consideration. In cereals, the husk and the pericarp are rich in pentosans, cellulose and lignin (Selvendran, 1984). On the other hand, the aleurone layer and the endosperm contain β-glucans and pentosans (Fincher and Stone, 1986). Botanically these ingredients contain the pericarp, testi and the aleurone layer (Fig. 2.3). During the milling process these two layers are separated from the endosperm (rich in starch and with a low concentration of fibre). However, part of the endosperm remains adhered to the external layers of the grain. Therefore the content of starch in the WB products may vary between different items of manufacture.

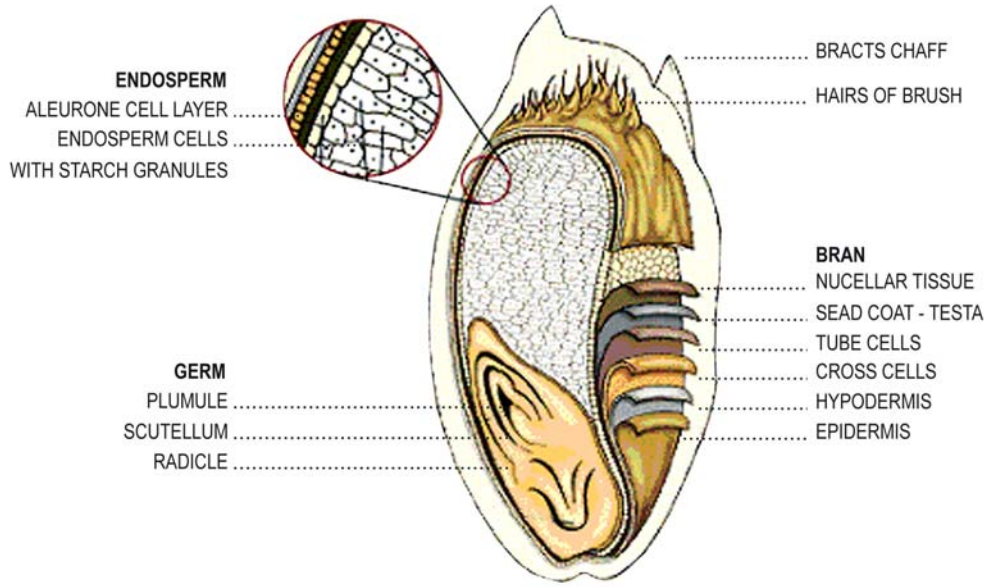


Fig. 2.3. Structure of the wheat kernel.

b) Resistant starch

Starch is the largest reserve polysaccharide in plants (Hizukuri, 1996). It is stored in insoluble granules in the endosperm. Its size and shape depends on the botanic species, it being small and with polyhedral shape in cereals (Greenwood, 1979). It contains two different types of polysaccharides: amylose and amylopectin (Table 2.5). Starches from cereals and tubers have approximately 25% amylose and 75% amylopectin (Eliasson and Gudmundsson, 1996). These linkages can be hydrolyzed by salivary α-amylase and pancreatic enzymes from the small intestine. The result of that break-down is: maltose (lineal chains of oligo-, tri- and disaccharides) and dextrins (branched oligosaccharides). These intermediary compounds are digested by carbohydrases situated in the epithelial surface of the intestinal villi (Low and Longland, 1990).

The hydrolysis of starch in the small intestine may be incomplete. Starch particles that escape from the intestinal digestion are called RS which is defined as "the starch and degradation by-products that resist intestinal enzymatic digestion in the small intestine and reach the large intestine of healthy animals where they are fermented" (Hogberg and Lindberg, 2006). Originally it has been classified in four different types:

- *RS type 1*: belongs to plants and food matrices like partial broken seeds (ex. legumes). The milling process of these products increases the availability of starch.
- *RS type 2*: granular starch, partially gelatinized and slowly hydrolyzed by α-amylase. Raw potatoes, green bananas and maize starch represent this group.
- *RS type 3*: backward starches: found in boiled rice and potatoes.
- *RS type 4*: chemically modified starches to improve its functional characteristics.

2.3. Host - dietary fibre - microbiota interactions

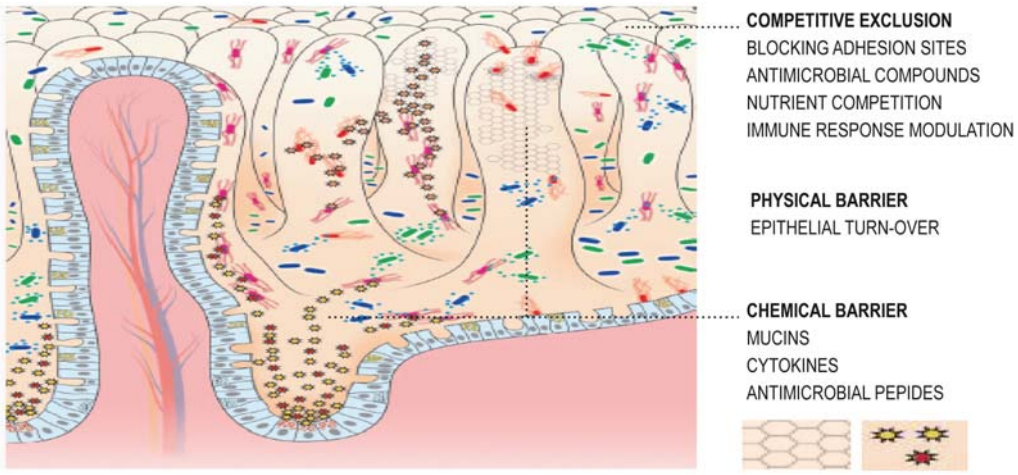
The presence of DF in the diet may modify the microbial equilibrium in the intestine and the physicochemical properties of digesta with a positive or detrimental impact on animal health and performance according to the level and source of the DF. In the upper parts of the small intestine the most notable effects of including fibre in the diet will result mainly from changes in the physicochemical properties of digesta and its beneficial effect resulting from its anti-adhesion capacity. By contrast, the changes promoted by fibre on the microbial composition in the large intestine are usually due to changes in the intestinal fermentation.

2.3.1. Interactions on the host's intestinal barrier

Components of the diet and the gut are in intimate contact within the intestinal tract, (Fig. 2.4). Thus, there is a dynamic balance between the host, the intestinal microbiota and the feed substrates that arrive in the GIT. The host participates in the mucus secretion, epithelial exfoliation, IgA production, peristaltic movements and the flow of digesta. On the other hand, bacteria collaborate in the secretion of metabolites that may inhibit the proliferation of other bacteria (bacteriocins). At the same time they compete for the nutrients and the intestinal receptors (Liebler et al., 1992). That capacity that the naïve microbiota have to avoid the colonization of the GIT by pathogenic bacteria is known as competitive exclusion (Fuller and Reeds, 1978; Van der Waaji, 1989). Although this phenomenon still remains controversial, scientists agree that both, bacteria and

host, are involved in the competitive exclusion (Hentges, 1986). As it will be described in this and subsequent paragraphs DF in the diet can play an important role in the competitive exclusion mechanism (blocking the bacterial adhesion), affecting the physical barrier or altering the chemical protection barrier.

Fig. 2.4. Diagram of the host intestinal protection mechanisms.



Several reports (Forstner and Fostner, 1994; Piel et al., 2007) have referred to the effects of DF on the mucus layer. Globet cells in the intestine secrete mucins and glycoproteins which are typical elements of the mucus layer. This layer protects the intestine against infections and physical, chemical and enzyme damages. At the same time, it helps to pass the luminal content through the GIT. For an optimal protection the mucus layer must be intact from a quantitative (thickness) and qualitative (composition of the sugars) point of view (Piel et al., 2007). This protective layer depends on the dynamic balance between the synthesis and secretion of mucus by the globet cells and the erosion of the mucus layer (Forstner and Fostner, 1994). The composition of the diet, particularly the level and the properties of the fibre ingredients, is an important element of this balance. Fibre inclusion can increase the excretion of mucins in the terminal ileum in monogastric animals (Leterme et al., 1998). This effect will be determined by the source and the particle size of the fibre rich ingredients. The inclusion of insoluble fibre like WB

increases the synthesis and secretions of mucins due to physical erosion (Hedemann et al., 2005). It also may be associated to the proteolytic breakage of the mucus layer (Schneeman et al., 1982). Depending on the monosaccharide content, mucins can be neutral or acidic (with CH chains rich in sulphates and sialic acids). The latter can be divided between sulphated and non-sulphated. The health status of the intestinal tract is related to the maturation degree of the intestinal mucins. The most mature mucins are sulphated (Van Leewen and Versantvoort, 1999). The presence of immature mucins in the intestinal lumen indicate a lower health status of the intestine associated with a higher need to renew the mucus layer due to the action of harmful agents (Fontaine et al., 1998).

It has been proposed that DF provokes an increase of synthesis and secretion of acidic mucins, raising the capacity of the mucus layer to resist the attack of bacterial enzymes, and favouring the excretion of intestinal pathogenic bacteria. On the other hand, a diet that increases the release of luminal mucins to the digesta due to its physical erosion will provide more substrate for the growth of intestinal bacteria (Montagne et al., 2003).

2.3.2. Modification of physicochemical properties and digesta transit time

As it has been described, part of the effects of the DF on the intestinal function is due to the modification of the physicochemical characteristics of the intestinal digesta. Some beneficial effects of DF have been associated to some of its physical and/or physicochemical characteristics, such as the effect on digesta transit time or hydration. However, some negative effects have also been described associated to an increase in digesta viscosity. Therefore it seems necessary to study the effects of DF on the hydration properties of digesta such as: swelling capacity, solubility and WRC.

Swelling capacity can be defined as the volume occupied by the fibre mass under the conditions studied. It can be measured by the method described by Kuniak and Marchessault (1972), which consist in weighing dry substrate in a glass cylinder and left overnight at 25ºC in excess water. Results are expressed as millilitres of swollen substrate per gram of starting DM.

WRC can be measured by different methods: centrifugation, filtration or dialysis bags (Thibault et al., 1992). The amount and type of NSP in the diet will determine its WRC. Increasing the level of soluble NSP (sNSP) in the feed will result in a higher hydration capacity. The consequences of the higher WRC of digesta will be: an increase of the volume of the GIT and a higher volume and weight of the faeces (Schneeman, 1999). A rise in the hydration properties of digesta will allow the microorganisms to enter into the cellular matrix enhancing NSP digestion (Auffret et al., 1993).

Viscosity is defined as the resistance of a fluid to move. It is usually measured in the supernatant obtained after the centrifugation of the intestinal digesta (Johansen et al., 1996). Normally, soluble fibre sources give a viscous digesta. The negative effects depend on the animal species considered. Poultry are much more susceptible to suffer this process compared to pigs (Bedford and Classen, 1992). A higher digesta viscosity has negative effects on the digestion and absorption of nutrients in the diet. At the same time, it also affects the intestinal structure. It increases the cell exfoliation in the apical parts of the intestinal villus causing an atrophy of those and an increase of crypts depth (Schiavon et al., 2004). Viscosity also may contribute to the proliferation of *E. coli* in the GIT. A higher viscosity digesta may slow-down the digesta transit, and facilitate proliferation of pathogenic bacteria. Therefore reducing the intestinal viscosity may contribute to the prevention of post-weaning diarrhoea. For this reason the incorporation in the diet of ingredients with a lower content of soluble β-glucans or arabinoxilans, or the inclusion of enzymes in the same ratio may improve the performance of the animals.

The physical effect of DF on the intestine is also an important factor. In particular, the bulking capacity of DF may increase the GIT motility and reduces the transit time in the entire GIT. It has been observed that the intestinal motility is penalized when animals are fed highly digestible diets. The ingestion of highly digestible diets may produce an atrophy of the intestinal mucosa that may be reversed with the inclusion of fibre in the diet (Goodlad and Wright, 1983). The effect of the DF on intestinal morphology and cellular turnover again depends on the fibre source and its level of inclusion in the diet. The inclusion of insoluble fibre such as straw in the diet for a period of 14 days in growing pigs increased the villus height

and the crypt depth in the ileum and the jejunum. At the same time, insoluble fibre also increased the number of mitosis in crypts of large intestine, cellular death and the DNA synthesis by the epithelial cells compared to a non-fibre diet (Jin et al., 1994). These results support the hypothesis that the inclusion of high levels of insoluble fibre in a diet increases the physical erosion on the mucosa and the cellular turnover in the jejunum, ileum and colon.

On the other hand, dietary fibres with a higher solubility together with a higher digesta viscosity are known to reduce the transit time by reducing the emptying of the stomach. The presence of pectins in the diet is usually associated with a decrease in the feed intake due to the increase on the luminal viscosity and the WRC of the intestinal digesta (Hedemann et al., 2006). Freire et al. (2000) reported that higher luminal viscosity causes an increase of digesta transit time producing a reduction of the feed intake in the post-weaning animals due to the feeling of satiety that this ingredient causes. In the same study the authors showed a decrease on the digesta transit time when an insoluble fibre based on alfalfa meal was included in the post-weaning diet compared to the soluble source (SBP).

2.3.3. Carbohydrates as anti-adhesive agents for infectious diseases

Another interesting effect of the DF in the small intestine is related to the ability of some CH to act as analogues of the intestinal bacterial receptors. As it has been described previously, the most common means of adhesion of numerous bacteria, are the surface lectins that combine with complementary CH present on the host surface (Sharon and Lis, 1989). In recent years, information has significantly increased on the compositional characteristics of glycosylated chains that are responsible for the adhesion of the most common strains of *E. coli* and their toxins (Table 2.6). The main conclusions of these studies suggest that the minimum sequences needed in the receptors for the *E. coli* K88 in the piglet are N acetyl hexosamine linked into β linkages; and terminals of galactose bound with β linkages on hexosamine residues (Grange et al., 2006).

Table 2.6. Fimbrial adhesins of enterotoxigenic *E. coli* and their adhesion carbohydrate factors.

Fimbria	Major carbohydrate adhesion factors	Reference
K88	Galβ(1-4)Glcβ(1-1)-cer	Grange et al., 2006
	Galβ(1-4)GlcNAcβ(1-3)Galβ(1-4)Glcβ(1-1)-cer	Grange et al., 2002
	GalNAcβ(1-4)Galβ(1-4)Glcβ(1-1)-cer	Grange et al., 2002
	Galβ(1-3)GalNAcβ(1-4)Galβ(1-4)Glcβ(1-1)-cer	Jin and Zhao, 2000
K99	NeuGc α2-3Galβ1-4Glcβ 1-1-cer	Kyogashima et al., 1989
987P	SO_3 Galβ1-1-cer	Dean-Nystrom et al., 1994
F18	α-fuc-(1-2)-β-Gal-(1-4)-GlcNAc	Snoeck et al., 2004

Considering that adhesion to the intestinal epithelium is a key stage in the pathogenesis of different diarrhoeas, it has been speculated that the use of analogues of the intestinal receptor could successfully block and prevent the GIT colonization by *E. coli*. In this way, several *in-vitro* studies have been carried out to test the ability of different substances or microorganisms (probiotics) to block the adhesion of some intestinal pathogens (Table 2.7).

Some authors suggest the use of CH as a blocking agent to prevent some intestinal infections. The objective of the anti-adhesion therapy is blocking or inhibiting the bacterial lectins by suitable CH or their analogues for the prevention and treatment of microbial diseases (Kahane and Ofek, 1996; Kelly and Younson, 2000). Saccharides are ideal for this purpose since many of those that inhibit bacterial adhesion are normal constituents of cell surfaces or body fluids such as milk. Moreover, since anti-adhesive agents do not act by killing or arresting the growth of the pathogens, it is very unlikely to cause the generation of strains resistant to such agents.

Table 2.7. Example of *in-vitro* inhibition test against enterotoxigenic *E. coli* in piglets.

Fimbria	Adhesion substrate	Blocking agent	Reference
K88	Piglet ileal mucus	*Lactobacillus spp.*	Blomberg et al., 1993
	Porcine mucus	Egg-yolk antibodies	Jin et al., 1998
	Porcine intestine	*L. grasseri**	Bogovic Matijasic et al., 2006
	Porcine mucus	*B. lactis*†, *L.rhamnosus*‡	Collado et al., 2007
K99	Porcine epithelium	Purified pilli	Isaacson et al., 1978
F18	Piglet intestinal villi	Monoclonal antibodies	Snoeck et al., 2004
	Caco-2 Cells	*Lactobacillus spp.*	Horosová et al., 2006

**L. grasseri, Lactobacillus grasseri; †B. lactis, Bifidobacterium lactis; ‡L.rhamnosus, Lactobacillus rhamnosus.*

In human nutrition, the search of CH mimetics that naturally occur in breast milk has been studied. Of particular interest in this respect are the fucosylated oligosaccharides (FucOS) that are effective inhibitors of the adhesion to human cells of the enteropathogen *Campylobacter jejuni*. Breast-fed infants suffer from a considerably lower incidence of diarrhoea than formula-feed infants (Morrow et al., 2005). In the same way, Lengsfeld et al. (2004) reported the anti-adhesive qualities of okra fruit against the adhesion of *Helicobacter pylori* to human gastric mucosa. This effect was assumed to be due to a combination of glycoproteins and highly acidic sugar compounds making up a complex three-dimensional structure that is fully developed only in the fresh fruit juice. Some studies in animals have also been performed using this strategy to prevent the occurrence of some bacterial diseases. Mouricout et al. (1990) reported that colostrum-deprived newborn calves infected with a lethal dose of *E. coli* K99 were cured by drinking water containing glycopeptides prepared from the non-immunoglobulin glycoporteins of cow plasma. In rabbits and infant rats, experimental pneumonia caused by *Streptococcus penumoniae* was markedly reduced by the intranasal or intratracheal administration of either free oligosaccharides or as neoglycoproteins (Idänpään-Heikkilä et al., 1997).

In animal nutrition, different studies showed the promising effects of glycoconjugates from different origins such as cramberry and blueberry extracts (Ofek et al., 1996), mannan-oligosaccharides (MOS) (Spring et al., 2000; Fernandez

et al., 2002), palm kernel extracts (Allen et al., 1997) or soya and fermented soya bean products (Kiers et al., 2002) to inhibit the adhesion of different pathogens such as *E. coli* or *Salmonella spp.*. In the same way, DF from plants seems suitable as alternative adhesion matrices because of their CH nature and low digestibility. It could be hypothesized that a host runs less risk of contracting a GIT infection when enteropathogenic bacteria adhere to DF instead of to the epithelial receptor cells. Once the bacteria are attached to the DF particle, it could be eliminated in the faeces. Using this strategy Kiers et al. (2002), Maiorano et al. (2007) and Becker et al. (2009) reported the positive effects of using soya beans, sesame seeds or pea hull extracts against *E. coli* K88 intestinal adhesion in post-weaning pigs. These results support the idea of testing whether some fibrous ingredients, particularly those rich in insoluble fibre such as WB or oat hulls, that had shown positive effects on the intestinal health in post-weaning pigs (Mateos et al., 2006) may have the ability of binding to the *E. coli* in the upper parts of the small intestine and so reduce the incidence of post-weaning diarrhoea.

2.3.4. Modification of the intestinal microbiota

The presence of an intestinal microbiota in the GIT provides different benefits to the host. It aids the feed digestion, produces trophic effects on the epithelium, stimulates the immune system and protects against pathogenic species (Savage, 1986; Liebler et al., 1992). In the post-weaning period the instauration of a healthy and stable microbiota in the GIT will be one of the keys for having successfully productive results at this stage. At this point, DF may again play an important role for its effect on the intestinal function and microbiota population. For that reason, different studies have reported different effects of DF inclusion on the intestinal ecosystem (Awati et al., 2006; Bikker et al., 2006; Hogberg and Lindberg, 2006; Wellock et al., 2007) depending on the fibre source (solubility and fermentability) and the period under consideration.

As it has been considered previously, there is a clear link between some soluble fibre sources and a likely increase in viscosity, an increased transit time and the proliferation of pathogenic bacteria in the early weaning period. McDonald et al. (1999) reported an increase on the haemolytic *E. coli* counts when guar gum was

incorporated in the post-weaning diet. On the other hand, there is also increasing agreement that the incorporation of an insoluble fibre source in the diet may have a beneficial effect in the intestine due to the stimulation of the intestinal transit. Thus, reducing the problems caused by the intestinal stasis observed in the post-weaning period (Hogberg and Lindberg, 2006; Mateos et al., 2006). However, after the first days of weaning, when the animal grows and acquires the ability to ferment CH, the incorporation of fermentable fibre in the diet can result in a beneficial effect due to the increase of the microbial population diversity. Konstantinov et al. (2004) observed an increase of the microbial intestinal diversity when SBP and FucOS were incorporated into the diet. Bikker et al. (2006) and Hermes et al. (2009) also described an increase of lactobacilli population and a reduction of the coliform population in the intestinal digesta of post-weaning pigs fed on a diet supplemented with WB and SBP. The beneficial effects were attributed to the fermentation of pectins from the diet. In addition, the interaction of fibre with the intestinal microbiota may lead to the production of SCFA, and the reduction of luminal pH. These changes may show antimicrobial effects against some pathogenic bacteria such as *E. coli* or *Salmonella spp*. At the same time, it can stimulate the proliferation of other bacteria that are not sensitive to acidic pH such as *Lactobacillus spp.* (Konstantinov et al., 2004; Bikker et al., 2006). Other studies demonstrated that the acid media in the intestinal tract may inhibit the proliferation of other pathogenic bacteria like *Clostridium difficile* (May et al., 1994).

2.3.5. Carbohydrate fermentation

Finally, one of the most beneficial interactions between DF, the host and the intestinal microbiota is the microbial fermentation of the substrates that reach the large intestine of pigs (Williams et al., 2001). The organic matter (OM) fermentation is a basic process that requires the contribution of different microbial groups related in the food chain (Wolin and Miller, 1983).

Again, the DF fermentability depends on different factors, such as:

- *Inclusion and composition of DF in the diet*: the composition of the diet is crucial in determining the composition and activity of the intestinal microbiota and thus the production of SCFA mixture and other end products that will be optimal for gut health. The digestibility of fat, proteins and starch in the small intestine reach around 80%, while DF only achieves 40-60%. This proportion may vary depending on the botanical type that we consider. The composition of the DF is also important. Non-cellulosic NSP usually has a higher fermentation compared to cellulose. At the same time, compounds with a higher solubility and WRC will ferment at a higher proportion (McBurney et al., 1985). Noblet and Bach Knudsen (1997) found that the digestibility of various fibre fractions in sows was higher for maize (0.74) compared to WB (0.46). Therefore, it is not only the level of DF that it is important, but also the type or the source of fibre plays a significant role in digestion and absorption. Feeding similar levels of either WB or SBP to pigs did not alter the absorption rate of glucose and amino nitrogen. However, SBP increased hindgut fermentation and, therefore, the absorption of SCFA; thus, indicating the source of fibre has an important effect (Michel and Rérat, 1998).

- *Feed processing*: the milling of feed ingredients will increase the digestibility due to a higher availability of the feed compounds to be digested by microbial or endogenous enzymes. At the same time, treating the ingredients with heat also resulted in a higher productivity of the animals (Herkelman et al., 1990). These positive effects related to the treatment of feed ingredients will be higher in post-weaning piglets (Van der Poel et al., 1990) compared to growing pigs due to the immaturity of the GIT of the former (Fadel et al., 1988). Ferguson and Harris (1997) demonstrated that the particle size of WB determined its fermentability in human studies. The WB with a higher particle size had a higher WRC. This resulted in an increase of the fermentation time compared to the WB diet with a smaller particle size.

- *Age and weight of the animals*: piglets weaned at 21 days old have a low enzymatic activity and a lower potential to digest nutrients than older animals (Noblet et al., 1994; Castillo et al., 2007b). With age, animals improve their capacity

to digest higher levels of fibre in the diet. Thus, Le Goff and Noblet (2001) propose the consideration of two energy values for each ingredient, especially fibrous ingredients, one for growing animals (under 60 kg) and one for adult animals (finishing pigs and sows).

In normal conditions, the intestinal bacteria hydrolyse the undigested and unabsorbed polysaccharides to their constituent sugars by means of a series of anaerobic energy-yielding reactions leading to the production of ATP which is used for bacteria basal and growth metabolism (Macfarlane and Cummings, 1991). The majority of the anaerobic bacteria of the large intestine use the glycolysis pathways that degrade glucose to pyruvate via the glucose-6-phosphate pathway (Prescott et al., 1996). Polysaccharides made of pentoses and pectins are first metabolised by the pentose phosphate pathway (MacFarlane and MacFarlane, 2003; Fig. 2.5) starting with the pentose to fructose-6-phosphate and glyceraldehyde-3-phosphate via xylulose-5-phosphate (Prescott et al., 1996). As shown in Fig. 2.4 the fermentation of the fibre diet gives as major products SCFA and gases (H_2, CO_2 and methane). The most important SCFA by percentage are: acetate, propionate, butyrate and organic acids such as lactate (Bach Knudsen et al., 1991; Ewing and Cole, 1994; Wang et al., 2004) which are known to play an important role in water absorption, pH control and the inhibition of pathogens.

The lactic and acetic acids are the most common end-products in the stomach and the small intestine while in the large intestine they are acetic, propionic and butyric (Bergman, 1990). The normal proportion of SCFA in an adult pig is: 65:27:8 (acetate: propionate: butyrate) (Cummings and Englyst, 1987). When the SCFA are produced they are absorbed by the colon epithelium and used as an energy source for maintaining the cellular function of the host (Cummings and Englyst, 1987). This absorption is favoured when the pH is low or when the SCFA concentration is high. Therefore, 95-99% of the total SCFA is absorbed before digesta arrives at the rectum (Von Engelhardt et al., 1989). In pigs the SCFA concentration in the large intestine may vary between 150 and 250 micromole/g FM, it being higher in the proximal parts of the large intestine (caecum and colon) and lower at the end of the large intestine.

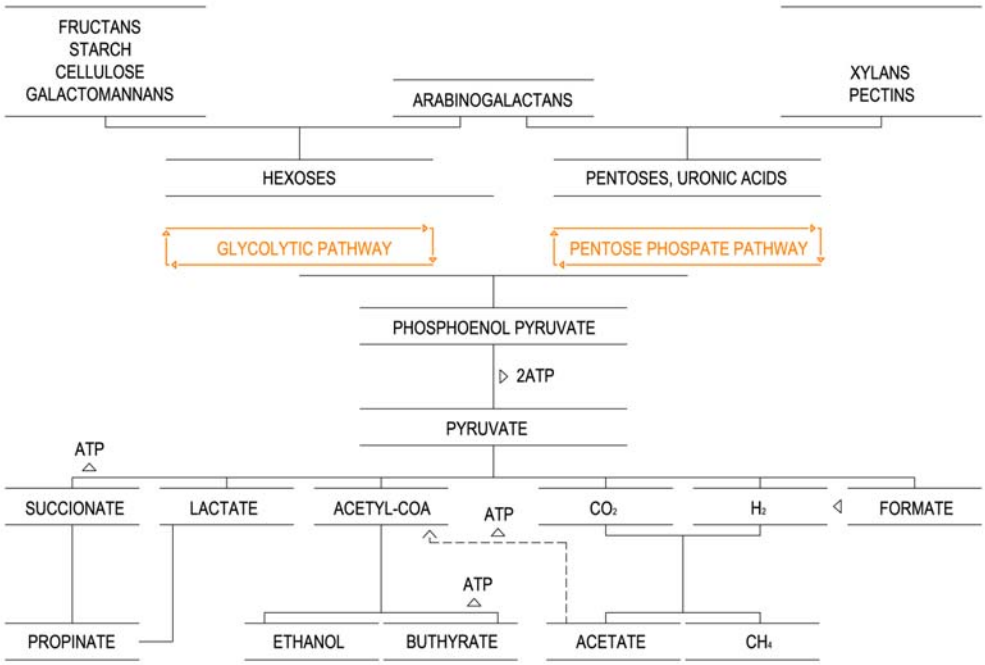


Fig. 2.5. Schematic representation of the pathways for polysaccharides fermentation in the pig intestines (Bindelle et al., 2008).

It has been described that SCFA may cover from 15 to 24% of the maintenance energy in growing pigs (Roediger, 1982). Acetate is transported to the liver and it is most used as a source of energy for muscle. Propionate is transformed to glucose in the liver. Butyrate does not pass into the blood but it is directly metabolised by the colonocytes to maintain metabolic activity and to stimulate the growth of the large intestine (Montagne et al., 2004; Hedemann and Bach Knudsen, 2007). Butyrate has been shown to regulate epithelial cell growth, to induce differentiation and apoptosis in the small intestine, to increase intestinal cell proliferation in piglets (Kien et al., 2007) and to improve digestive and absorptive capacities of the small intestine in pigs (Claus et al., 2007). In general it is accepted that a collateral consequence of the CH fermentation is the inhibition of the protein fermentation in the GIT (Awati et al., 2006).

CHAPTER 3

Objectives and Experimental Design

The ban of AGP inclusion in feed has caused an increase in interest of studying alternative strategies. The project AGL2005-07438-C02-01 entitled: "Feeding strategies for enhancing a healthy gut in early weaned pigs", focused the study on the the immaturity of the GIT of animals around weaning, and microbiota and intestinal health as affected by the inclusion of DF in weaned pigs. The project has been carried out by the Grup de Nutrició Animal del Departament de Ciència Animal i dels Aliments and in cooperation with the University of Manitoba (Winnipeg, Canada). This thesis accounts for part of the work carried out in this project, fixing the following as its main objectives:

1. To evaluate the interest of incorporating a source of fibre in the post-weaning diets in order to promote the maturation of the digestive tract and the establishment of a healthy microbiota to minimize the occurrence of intestinal disorders.

2. To evaluate the effect of fibre inclusion on the performance, physicochemical properties of digesta as well as the effect on the activity and composition of the intestinal microbiota.

3. To study the likely mechanisms by which fibre may modify the intestinal microbiota, and develop standard methodologies to carry out these studies.

4. To evaluate the effect of fibre inclusion in the piglet diets when an antimicrobial, such as ZnO, is simultaneously included in the diet.

To assess these four objectives, four different trials were designed. Results will be included in chapters 4 to 7.

In Trial 1 (Molist et al., 2009a), the effects of increasing the NSP content of a piglet diet by using moderate amounts of either a more insoluble (WB) and/or a more soluble (SBP) NSP source on nutrient utilization, the physicochemical

properties of digesta, and microbial activity and populations of pigs around weaning were analyzed.

Trial 2 (Molist et al., 2009b) was designed with the aim of studying the effects of WB inclusion and particle size of WB on the microbial composition in the digesta and intestinal mucosa of newly weaned pigs challenged with enterotoxigenic *Escherichia coli* $K88^+$.

Trial 3 (Molist et al., 2010a) aimed to confirm the likely beneficial effects of including WB in the diet of early weaned piglets and to assess which, if any, of the productive, digestive or microbial effects of WB are dominant in changing the digesta transit time.

Trial 4 (Molist et al., 2010b) was designed to evaluate: 1.- the likely role of WB and other fibre sources on their ability to bind *E. coli* in-vitro (Experiment 1). 2.- the effects of including WB and/or ZnO in the diet of newly weaned piglets on the productive performance and the microbial activity in the GIT (Experiment 2); and finally 3.- the likely interactions which may be established *in-vitro* between the WB and ZnO in the intestinal digesta and with respect to the *E. coli* growth (Experiment 3).

CHAPTER 4

"Effects of the insoluble and soluble dietary fibre on the physicochemical properties of digesta and the microbial activity in early weaned piglets".

Animal Feed Science and Technology (Molist et al., 2009a)
Accepted

Abstract

The aim of this work was to asses the influence of including two different sources of non-starch polysaccharides (NSP): insoluble NSP (iNSP) like wheat bran (WB) and/or soluble NSP (sNSP) like sugar beet pulp (SBP) on the nutrient digestibility and the physicochemical characteristics of the hindgut digesta, and on the microbial population and the fermentation end-products. A total of 32 piglets (7.4 ± 0.76 kg of body weight (BW)) were distributed into four experimental diets: a control diet (CT), or diets with 8% WB, 6% SBP, or 4% WB and 3% SBP (WB-SBP). Two experimental periods were considered (0-10 and 10-15 days after weaning) during which BW and voluntary feed intake were measured. Four animals per treatment were euthanized on days 10 and 15. Colon digesta was sampled and analyzed for organic matter digestibility (OMd) and starch digestibility, unbound water, water retention capacity (WRC) and short chain-fatty acids (SCFA). At the same time, enterobacteria and lactobacilli loads were determined in caecum digesta. The presence of iNSP in the diet (WB and WB-SBP diets) diminished the unbound water of colonic digesta in the two experimental periods ($P = 0.01$ on day 10, and $P < 0.05$ on day 15) and increased the butyric acid concentration ($P < 0.05$) on day 15, compared to the CT diet. Including iNSP and sNSP in the same diet (WB-SBP) decreased ($P < 0.05$) the enterobacteria counts on caecum digesta on day 15 compared to the CT diet indicating a synergistic effect of the two different sources on the microbial population. Consumption of diets with higher iNSP content, or the combination of iNSP+sNSP in the early weaning period modifies physicochemical characteristics and affects the microbial colonization and fermentation patterns in the hindgut.

4.1. Introduction

Weaning is a stressful period for piglets, often associated with reduced feed intake, little or no weight gain, and marked changes in the structure and function of the gastrointestinal tract (GIT) (Boudry et al., 2004). The temporary low capacity of piglets to acidify gastric contents, the accumulation of undigested feed in the small intestine and the protein fermentation in the hindgut are all factors involved in the proliferation of pathogenic bacteria (Lallès et al., 2007). Piglet diets are low in fibre because it is believed that fibre reduces digestibility and feed intake (Eggum, 1995). However, there is growing evidence that increasing the dietary non-starch polysaccharides (NSP) content may reinforce commensal microbiota in the hindgut by increasing carbohydrate fermentation instead of protein (Williams et al., 2001). Moreover, significant reductions in the counts of coliform bacteria and the incidence of diarrhoea have been described particularly when insoluble NSP such as oat hulls are included in low fibre diets (from 20 to 40 g/kg) (Mateos et al., 2006). Similar results have been observed when soluble, non-viscous NSP such as lactose or inulin (Pierce et al., 2007; Wellock et al., 2008), and sugar beet pulp (SBP), wheat bran (WB) and raw native starch (Bikker et al., 2006) are included in the diet. Wellock et al. (2008) have suggested that NSP in weaned diets that do not increase digesta viscosity may have a beneficial effect on gut health. It could be hypothesized that the beneficial or detrimental effects of an increased NSP content in the diet will depend on its composition and physicochemical properties. The aim of the present study was to evaluate what are the effects of increasing the NSP content of a piglet diet by using moderate amounts of either a more insoluble (WB) or/and a more soluble (SBP) NSP source on the nutrient utilization, physicochemical properties of digesta, and microbial activity and populations of pigs around weaning.

4.2. Materials and methods

4.2.1. Animals and diets

This experiment was performed at the Experimental Unit of the Universitat Autònoma de Barcelona and received prior approval from the Animal Protocol Review Committee of this institution. A total of 32 commercial crossbred piglets

([Large White x Landrace] x Pietrain), which had been excluded from receiving creep feed, were weaned at 24 days of age with an average BW of 7.4 ± 0.76 kg.

Pigs were allotted into 16 pens (2 animals/pen) and distributed to four experimental treatments (Table 4.1). The dietary treatments included a control diet (CT) based on ground corn, barley, and soybean protein concentrate, and three NSP supplemented diets formulated to be isoenergetic (14.25 MJ/ kg, metabolizable energy), and isoproteic (CP, 190 g/kg; total Lys, 14.5 g/kg). Similar increases on the content of NSP were obtained by replacing corn and barley with either 8% of WB, 6% of SBP, or 4% of WB plus 3% of SBP (WB-SBP). Diets contained 0.15% of Cr_2O_3 as a digestibility marker.

4.2.2. Experimental procedures and sampling

Animals received the diets from days 1 to 15 of the experiment. Two experimental periods (0-10 days and 10-15 days after weaning) were used to evaluate the adaptation of the gut to the experimental diets. On day 10 and 15, the heaviest animal of each pen was euthanized with an intravenous injection of sodium pentobarbital (200 mg/kg BW). Animals were bled, and the abdomen was immediately opened to tie and remove the caecum and the colon which were emptied and sampled. Samples of about 1 g of digesta from the caecum were kept in weighed tubes and immediately frozen at -80ºC for microbial counts analyses. Samples from the colon consisted in a pool of entire colon contents. Half of the collected samples was freeze-dried and then dried at 103ºC for complete water removal. The other half was divided into three aliquots: 3 g was collected into previously weighed 10 mL screw cap tubes for water retention capacity (WRC) analysis; the remainder was collected in tubes for unbound water and short-chain fatty acids (SCFA) analyses.

Table 4.1. Composition and chemical analysis of pre-starter diets (g/kg dry matter)

	Diets[a]			
	CT	WB	SBP	WB-SBP
Ingredients				
Corn	330	279	299	288
Barley	238	210	210	210
Whey	130	130	130	130
High fat whey	100	100	100	100
Soybean protein concentrate	92	87	90	90
Wheat gluten	30	30	30	30
Fish meal LT[b]	40	40	40	40
Wheat bran	-	80	-	40
Sugar beet pulp	-	-	60	30
Sunflower oil	-	6.5	3.0	4.0
Calcium carbonate	11.7	10.6	10.1	9.7
Dicalcium phosphate	9.1	8.4	9.2	9.0
Synthetic aminoacids[c]	12.2	12.2	12.2	12.2
Vitamin and mineral premix[d]	7.8	7.8	7.8	7.8
Chemical analysis				
Dry matter	903	903	902	903
Gross energy (MJ/Kg)	19.8	19.7	19.7	19.7
Crude protein (CP; N x 6.25)	231	233	231	231
Starch	394	347	360	350
Neutral detergent fibre	77	96	90	95
Acid detergent fibre	17	21	25	24
Total NSP[e]	102	138	145	139
Insoluble NSP[f]	84	117	116	114
Soluble NSP[g]	18	21	29	25
Arabinoxylans	32	51	46	47
Uronic acids	8	7	14	12
Ether Extract	78	83	76	81
Ash	64	66	66	66

[a]Experimental diets: CT, control diet; WB, wheat bran diet; SBP, sugar beet pulp diet and WB-SBP, wheat bran and sugar beet pulp diet.
[b]Fish meal low temperature: product obtained by removing most of the water and some or all of the oil from fish by heating at low temperature (<70ºC) and pressing.
[c]Synthetic aminoacids: L-Lysine 0.99, DL-Metionine 0.99, L-Triptophan 0.10, L-Threonine 0.98.
[d]Supplied per kilogram of feed: 5000 IU of vitamin A, 1000 IU of vitamin D3, 15.0 mg of vitamin E, 1.3 mg of vitamin B1, 3.5 mg of vitamin B2, 1.5 mg of vitamin B6, 0.025 mg of vitamin B12, 10.0 mg of calcium pantothenate, 1.3 g of coline chloride, 15.0 mg of niacin, 15.0 mg of biotin, 0.1 mg of folic acid, 2.0 mg of vitamin K3, 80.0 mg of Fe, 6.0 mg of Cu, 0.7 mg of Co, 60.0 mg of Zn, 30.0 mg of Mn, 0.7 mg of I, 0.1 mg of Se, 0.15 mg of etoxiquin and 1.5 g of chromic oxide.
[e]Non-starch polysaccharides.
[f]Insoluble non-starch polysaccharides.
[g]Soluble non-starch polysaccharides.

4.2.3. Analytical procedures

Chemical analyses of the diets (Table 4.1) were performed according to the Association of Official Analytical Chemists standard procedures (AOAC, 1995). Total starch, total soluble and insoluble NSP, arabynoxilans and uronic acid content were analyzed in feed using a modification of the Uppsala procedure (Theander and Aman, 1979) described by Bach Knudsen (1997). Chromium III oxide concentration in feed and digesta was determined by atomic absorption spectrophotometry following the method of Williams et al. (1962). WRC of fresh colon digesta contents was determined by centrifugation (2500 x g, 25 min) following the procedure of Anguita et al. (2007), and unbound water was determined as the percentage of the liquid phase obtained after fresh colon digesta was left to stand for 3 h at room temperature in a test tube. The DNA from caecum digesta was extracted and purified using the commercial QIAamp DNA Stool Mini Kit (Qiagen, West Sussex, UK), and enterobacteria and lactobacilli were quantified by real-time PCR using SyBR Green dye following the procedure of Castillo et al. (2006). Short chain fatty acids and lactic acid were determined as described by Jensen and Jorgensen (1994).

4.2.4. Statistical analyses

Data were subjected to ANOVA, with dietary treatment as the classification factor, using the GLM procedure (SAS Inst., Inc., Cary, NC, USA). Pen was considered the experimental unit (n = 4). Data of productive performance was studied including initial BW as a covariate. The effect of the day of killing was studied for some parameters including time in the model as a classification factor. The alpha level used for the determination of significance for all the analysis was 0.05 and trends (alpha < 0.10) are also reported.

4.3. Results and discussion

The effects of NSP content on feed intake, growth performance and fractional OM digestibility (OMd) and starch digestibility on the colon are shown in Table 4.2.

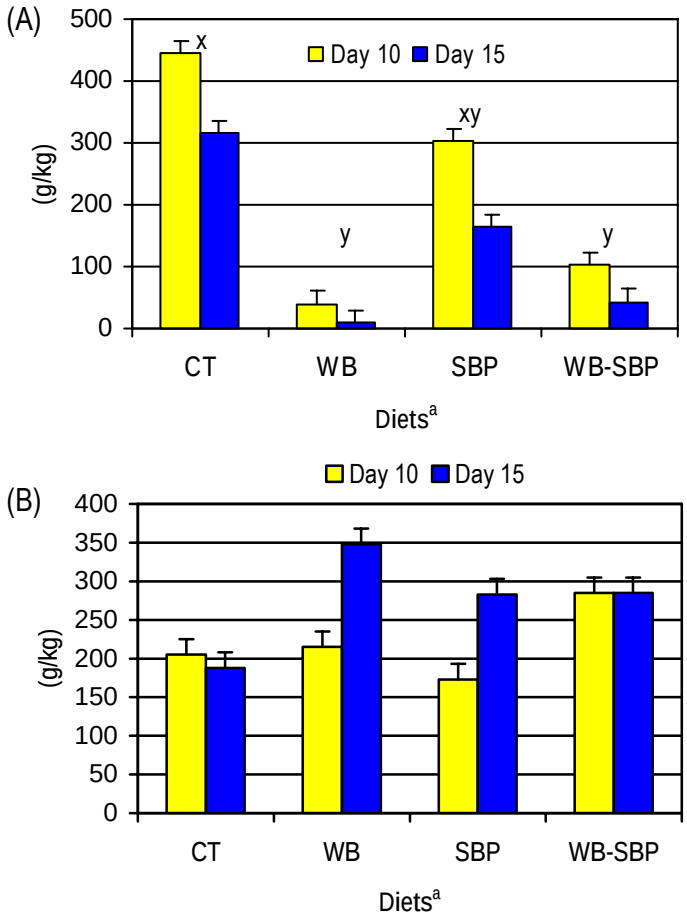


Fig. 4.1. Unbound water (A) and WRC (B) of colonic digesta on piglets fed experimental diets. [a] Diets: CT, control diet; WB, wheat bran diet; SBP, sugar beet pulp diet; and WB-SBP, wheat bran and sugar beet pulp diet. Values are least square means and standard error of the mean (n=4). The P-values for diets was 0.003 and 0.03 in day 10 and 15 respectively; and the P-value for period (0-10 and 10-15 days) was 0.10. Different superscripts (x and y) denote significant difference between diets ($P < 0.05$). (B) The P-value for diets were 0.07 and 0.09 in day 10 and 15 respectively; and the P-value for period (0-10 and 10-15 days) was 0.03.

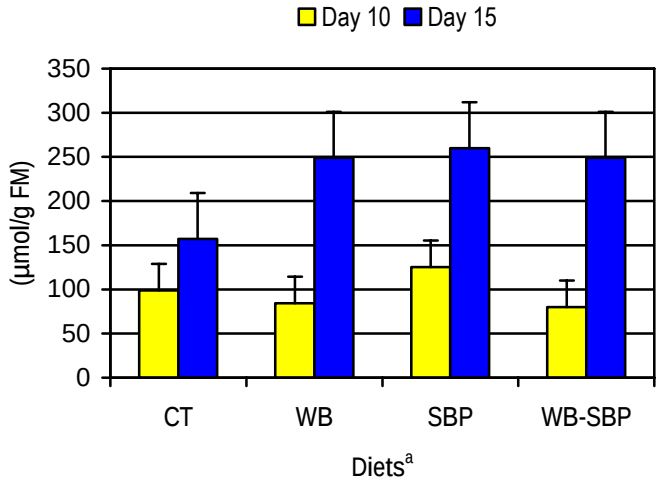


Fig. 4.2. Total SCFA concentration in colonic digesta in early weaned piglets. [a]Diets: CT, control diet; WB, wheat bran diet; SBP, sugar beet pulp diet; and WB-SBP, wheat bran and sugar beet pulp diet. Values are least square means and standard error of the mean (n=4). The P-values for diets were 0.173 and 0.115 in day 10 and 15 respectively; and the P-value for period (0-10 and 10-15) was 0.001.

Animals fed the WB diet showed a higher ADFI ($P < 0.05$) and tended to have a higher ADG ($P = 0.10$) compared to the CT diet from days 0 to 10. Quantitative but not significant differences between WB and CT were still observed from days 10 to 15 probably because of the high variability of the results. The OMd on colon digesta was higher in the SBP diet ($P = 0.001$) compared to the other three experimental diets on day 15. However, no significant differences were observed between diets in the OMd on day 10 or in the starch digestibility in any of the experimental periods. These results agree well with those referred by Högberg and Lindberg (2004) who found an increased daily gain of the piglets when NSP was increased (from 106 to 197 g/kg). The authors suggested that the increased weight gain in the high NSP diets was most likely caused by a higher weight gain of internal organs. Similar increases have also been observed by Mateos et al. (2006) when oat hulls, an insoluble and highly lignified source of fibre, were incorporated, especially in rice-based diets. The authors suggested that the insoluble DF might influence motility

and transit time of digesta, and recommend the use of diets containing about 60 g NDF/kg diet for piglets from 6 to 12 kg LW.

Table 4.2. Average daily feed intake (ADFI), average daily gain (ADG), coefficient of total tract apparent organic matter (OMd) and starch digestibility (STd) in early weaned pigs

	Diets[a]				SEM	P-diet
	CT	WB	SBP	WB-SBP	(n = 4)	
ADFI (g/animal and day)						
0 – 10 days	199 [y]	306 [x]	295 [xy]	218 [xy]	46.7	<0.05
10 – 15 days	222	361	317	323	128.7	0.492
ADG (g/animal and day)						
0 – 10 days	-2.9	68.5	34.1	-23.1	76.74	0.101
10 – 15 days	36.9	216.9	181.0	220.0	174.47	0.442
OMd						
0 – 10 days	0.816	0.780	0.702	0.760	9.87	0.371
10 – 15 days	0.786 [y]	0.786 [y]	0.832 [x]	0.802 [y]	0.0126	<0.01
STd						
0 – 10 days	0.883	0.889	0.783	0.888	0.0584	0.133
10 – 15 days	0.906	0.882	0.886	0.887	0.0208	0.482

Different superscripts (x and y) in the same row denote significant difference (P < 0.05).
[a]Diets: CT, control diet; WB, wheat bran diet; SBP, sugar beet pulp diet; and WB-SBP, wheat bran and sugar beet pulp diet.

The experimental diets were designed to deliver a range in the amount (from 77 to 96 g NDF/kg or 102 to 145 g NSP/kg) and nature (insoluble and soluble) of NSP in the diet. The WB (the coarse outer membrane of the wheat kernel) was chosen because of its higher proportion of NSP as insoluble cellulose and arabinoxylans (Bach Knudsen, 1997), whereas the SBP contains a higher proportion of NSP as soluble pectins. Differences between soluble and insoluble NSP have been shown to influence the digestive processes in the growing pig. While soluble fibre tends to be highly fermentable in nature (Bach Knudsen, 2001), insoluble fibre may increase the WRC and provide a substrate that is slowly fermented by the microflora in the distal intestine (Freire et al., 2000). Data related to unbound water and WRC on the colonic digesta is presented in Fig. 4.1. The inclusion of WB in the diets diminished the percentage of unbound water (Fig. 4.1A) on the colonic digesta in the two experimental periods (P = 0.01 on day 10 and P < 0.05 on day 15)

compared to the CT diet. The WRC (Fig. 4.1B) of the digesta tended also to be affected by the experimental diets, showing the highest values for pigs fed on WB and the lowest values occurring with the SBP diet on day 10 and the CT diet on day 15. Our results demonstrate the high influence of the NSP content and composition on the physicochemical properties of digesta. The main effects were observed with WB and may reflect the higher water-binding capacity of the insoluble long-chain NSP as compared to the low WRC of other digesta compounds, such as the starch or protein (Anguita et al., 2007).

Table 4.3. Concentration (µmol/ g FM) of short-chain fatty acid (SCFA) and lactic acid on colon digesta and bacterial populations (enterobacteria and lactobacilli) measured by real-time PCR (log 16S rDNA gene copies/ g FM) on caecum digesta of piglets 15 days after weaning

	Diets[a]				SEM	P-diet
Item	CT	WB	SBP	WB-SBP	(n = 4)	
Formic	0.9	1.2	1.1	1.1	0.41	0.924
Acetic	68.2	109.2	144.7	122.9	35.95	0.172
Propionic	45.5	54.2	65.9	76.9	20.82	0.234
Butyric	11.7 ^{y}	35.9 ^{x}	12.2 ^{y}	31.3 ^{x}	10.83	0.027
Isoacids	0.5	0.5	0.4	0.7	0.29	0.615
Succinic	7.1	0.2	0.5	0.2	7.73	0.547
Lactic	20.8	34.6	54.7	6.7	33.18	0.329
Enterobacteria	11.1 ^{x}	10.0 ^{xy}	10.8 ^{xy}	8.3 ^{y}	1.14	<0.05
Lactobacilli	11.7	12.0	11.9	11.5	0.53	0.572

Different superscripts (x and y) in the same row denote significant difference (P < 0.05).
[a]Diets: CT, control diet; WB, wheat bran diet; SBP, sugar beet pulp diet; and WB-SBP, wheat bran and sugar beet pulp diet.

The concentrations of SCFA in the colonic digesta and the enterobacteria and lactobacilli counts in caecum digesta are shown in Fig. 4.2 and Table 4.3. A pronounced increase (P < 0.01) in the SCFA concentration was observed from days 10 to 15, especially in diets containing WB and/or SBP. The increases on the SCFA concentration with the NSP diets could be associated with a higher WRC of digesta, which has been used as a predictor of the degradability of the DF (Auffret et al., 1993; Drochner et al., 2004), but also with the higher feed intake observed in animals fed on the NSP supplemented diets. Among NSP supplemented diets, WB and WB-SBP promoted an increase in the amount of butyric acid (P = 0.027) on day 15 compared to SBP and CT diets. No significant differences were observed

between experimental treatments in the SCFA profile on day 10. A similar increase on the butyrate percentage has been observed recently by Högberg and Lindberg (2006), and Bikker et al. (2006) in newly weaned pigs fed on diets supplemented with higher amounts of cereals and WB (from 109 to 203 g NSP/kg), or WM (4%), potato starch (5%) and SBP (4%) (from 112 to 165 g NSP/kg). From this respect, butyrate is considered an important metabolite because it is the principal oxidative fuel for the colonocytes and may have beneficial tropic effects on inflamed caeco-colonic mucosa (Topping and Clifton, 2001). It is accepted that starch and bran from wheat or oat, stimulate the formation of butyrate (Bach Knudsen et al., 1993), while xylans and pectin rich fractions (SBP) are all associated with a relatively low formation of butyrate (Anguita et al., 2007). Lactate produced by lactic acid bacteria has also been recently suggested as a major precursor for butyrate synthesis (Bourriaud et al., 2005), with butyrate formation increasing with decreased transit time in the GIT (Lewis and Heaton, 1997) or higher dilution rates *in vitro* (Sharp and MacFarlane, 2000). From this respect, the effect of WB on reducing transit time is well established (Bardon and Fioramonti, 1983). Changes in the fermentation pattern between diets were associated with changes in the enterobacteria counts on day 15, with pigs fed on WB-SBP presenting the lowest counts. No significant differences were observed on the enterobacteria population on day 10 and on the counts of lactobacilli on day 10 and 15. Similar results have been previously described by Bikker et al. (2006) when SBP, WB and raw native starch were simultaneously included in the diet. This indicates that a proper combination of different NSP sources may improve the microbial status of the young piglets.

4.4. Conclusion

Based on the results of the present study, it can be concluded that an increase in the amount of NSP in the diet may enhance the fermentation activity in the large intestine of piglets after weaning. Diets with a higher amount of insoluble NSP or a combination of insoluble and soluble NSP promoted a beneficial shift in the microbial colonization, with a higher production of butyric acid in the large intestine and lower enterobacteria counts in the digesta.

4.5. Acknowledgments

This research was supported by the Spanish CICYT (project AGL2005-07438-C02-01). We thank Ministerio de Educación, Cultura y Deporte, Spain for research fellowships.

4.6. References

Anguita, M., Gasa, J., Nofrarias, M., Martín-Orúe, S.M., Pérez, J.F., 2007. Effect of coarse ground corn, sugar beet pulp and wheat bran on the voluntary intake and physicochemical characteristics of digesta of growing pigs. Livest. Sci. 107, 182-191.

AOAC, 1995. In: Association of Official Analytical Chemists (Ed.), Official Methods of Analysis. Arlington, VA, USA.

Auffret, A., Barry, J. L., and Thibault, J. F., 1993. Effect of chemical treatments of sugar-beet fibre on their physicochemical properties and on their *in vitro* fermentation. J. Sci. Food Agric. 61, 195-203.

Bach Knudsen, K. E., Jensen, B. B., Hansen, I., 1993. Digestion of polysaccharides and other major components in the small and large intestine of pigs fed diets consisting of oat fractions rich in ß-D-glucan. Br. J. Nutr. 70, 537-556.

Bach Knudsen, K.E., 1997. Carbohydrate and lignin contents of plant materials used in animal feeding. Anim. Feed Sci. Technol. 67, 319-338.

Bach Knudsen, K.E., 2001. The nutritional significance of “dietary fiber” analysis. Anim. Feed Sci. Technol. 90, 3-20.

Bardon, T., Fioramonti, J., 1983. Nature of the effects of bran on digestive transit time in pigs. Br. J. Nutr. 50, 685-690.

Bikker, P., Dirkzwager, A., Fledderus, J., Trevisi. P., Le Huërou-Luron, I., Lallès, J.P., Awati, A., 2006. The effect of dietary protein and fermentable carbohydrates levels on growth performance and intestinal characteristics in newly weaned piglets. J. Anim. Sci. 84, 3337-3345.

Boudry, G., Péron, V., Le Huërou-Luron, I., Lallès, J.P., Sève, B., 2004. Weaning induces both transient and long-lasting modifications of absorptive, secretory, and barrier properties of piglet intestine. J. Nutr. 134, 2256-2262.

Bourriaud, C., Robins, R.J., Martin, L., Kozlowski, F., Tenailleau, E., Cherbut, C., Michel, C., 2005. Lactate is mainly fermented to butyrate by human intestinal microfloras but inter-individual variation is evident. J. Appl. Microbiol. 99, 201-212.

Castillo, M., Martín-Orúe, S.M., Roca, M., Manzanilla, E.G., Badiola, I., Pérez, J.F, Gasa, J., 2006. The response of gastrointestinal microbiota to avilamycin, butyrate, and plant extracts in early-weaned pigs. J. Anim. Sci. 84, 2725-2734.

Drochner, W., Kerler, A., Zacharias, B., 2004. Pectin in pig nutrition, a comparative review. J. Anim. Physiol. Anim. Nutr. 88, 367-380.

Eggum, B.O., 1995. The influence of dietary fibre on protein digestion and utilization in monogastrics. Arch. Anim. Nutr. 48, 89–95.

Freire, J.P.B., Guerreiro, A.J.G., Cunha, L.F., Aumaitre, A., 2000. Effect of dietary fibre source on total tract digestibility, caecum volatile fatty acids and digestive transit time in the weaned piglet. Anim. Feed Sci. Technol. 87, 71-83.

Högberg, A., Lindberg, J.E., 2004. Influence of cereal non-starch polysaccharides and enzyme supplementation on digestion site and gut environment in weaned piglets. Anim. Feed Sci. and Technol. 116, 113-128.

Högberg, A., Lindberg, J.E., 2006. The effects of level and type of cereal non-starch polysaccharides on the performance, nutrient utilization and gut environment of pigs around weaning. Anim. Feed Sci. and Technol. 127, 200-219

Jensen, B.B., Jorgensen, H., 1994. Effect of dietary fiber on microbial activity and microbial gas production in various regions of the gastrointestinal tract of pigs. Appl. Environ. Microbiol. 60, 1897-1904.

Lallès, J.P., Bosi, P., Smidt, H., Stokes, C.R., 2007. Weaning – a challenge to gut physiologists. Livest. Sci. 108, 82-93.

Lewis, S.J., Heaton, K.W., 1997. Increasing butyrate concentration in the distal colon by accelerating intestinal transit. Gut. 41(2), 245-51.

Mateos, G.G., Martín, F., Latorre, M.A., Vicente, B., Lazaro, R., 2006. Inclusion of oat hulls in diets for young pigs based on cooked maize or cooked rice. Anim. Sci. 82, 57-63.

Pierce, K.M., Callan, J.J., McCarthy, P., O´Doherty, J.V., 2007. The interaction between lactose level and crude protein concentration on piglet post-weaning

performance, nitrogen metabolism, selected faecal microbial populations and faecal volatile fatty acid concentrations. Anim. Feed Sci.Technol. 132, 267-282.

Sharp, R., Macfarlane, G.T., 2000. Chemostat enrichments of human feces with resistant starch are selective for adherent butyrate-producing Clostridia at high dilution rates. Appl. Environ. Microbiol. 66, 4212-4221.

Theander, O., Aman, P., 1979. Studies on dietary fibre. I. Analysis and chemical characterization of water-soluble and water-insolube dietary fibres. Swed. J. Agric. Rev. 9, 97-106.

Topping, D.L., Clifton, P.M., 2001. Short-chain fatty acids and human colonic function: Roles of resistant starch and nonstarch polysacharides. Physiol. Rev. 81, 1031-1064.

Wellock, I.J., Fortomaris, P.D., Houdijk, J.G.M., Wiseman, J., Kyriazakis, I., 2008. The consequences of non-starch polysaccharide solubility and inclusion level on the health and performance of weaned pigs challenged with enterotoxigenic *Escherichia coli*. Br. J. Nutr. 99, 520-530.

Williams, B.A., Verstegen, M.W.A., Tamminga, S., 2001. Fermentation in the large intestine of single-stomached animals and its relationship to animal health. Nutr. Res. Rev.14, 207-227.

Williams, C.H., David, D.J., Lismaa, O., 1962. The determination of chromic oxide in feces samples by atomic absorption spectrophometry. J. Agric. Sci. 59, 381-385.

CHAPTER 5

"Effect of wheat bran on the health and performance of weaned pigs challenged with *Escherichia coli* K88^{+}".

Livestock Science (Molist et al., 2009b)
Accepted

Abstract

The leading cause of post-weaning diarrhoea in pigs is *Escherichia coli*. Previous studies showed that inclusion of wheat bran (WB) in the diet of weaned pigs decreased number of pathogenic *E. coli* in the faeces and reduced the incidence of post-weaning diarrhoea. It is not clear whether it is the WB alone that improves gut health, or whether it is the particle size of the WB that is important. In this experiment we used an *E. coli* K88$^+$ challenge model to test the importance of supplementing WB and particle size of the WB. A total of 36 individually-housed piglets (17 ± 0.77 d) were assigned randomly to one of four experimental groups. Treatments were: (1) a negative control diet (NC) based on corn, wheat, barley and soybean meal; (2) NC + 4% coarsely milled WB (WBc, 1088 µm); (3) NC + 4% finely milled WB (WBf, 445 µm); and (4) a positive control diet (PC) consisting of the NC diet supplemented with a commercial feed grade antibiotic mix. At 26 d of age, pigs were experimentally infected with 6.2 x 10^9 cfu/ml of *E. coli* K88$^+$. Body weight, feed intake, and diarrhoea were monitored. Pigs were euthanized 7 d after infection. Ileal digesta and mucosa were taken for *E. coli* enumeration and for determination of SCFA and indices for richness and diversity of microbiota. There were no significant differences in ADG, ADFI, G:F ratio attributable to dietary treatment. Inclusion of WB, either fine or coarse, significantly ($P < 0.05$) decreased *E.coli* numbers in the ileal digesta. The use of WBc had an additional benefit because the *E. coli* K88$^+$ numbers were significantly lower ($P < 0.05$) and the SCFA in ileal digesta was higher ($P < 0.05$) compared to WBf. We conclude that both WB per se, and the particle size of WB have an effect on gut health in weaned pigs.

5.1. Introduction

Post-weaning diarrhoea is a multifactorial disease provoked sometimes by certain strains of *Escherichia coli* and its expression is influenced by the diet (Hampson, 1994). Some authors have reported that inclusion of fermentable carbohydrates in weaner pig diets may decrease post-weaning collibacilosis (PWC) by promoting proliferation of commensal microbiota and by decreasing protein fermentation in the digestive tract (Awati et al., 2006). In a recent experiment, we observed that inclusion of wheat bran (WB) in the diet of piglets from week 1 to 2 after weaning decreased the pathogenic *E. coli* numbers in the colon reducing the incidence of post-weaning diarrhoea (Molist et al., 2009a). However it was not clear whether WB decreased PWD by modulating the microbial activity in the small intestine or through changes on the physicochemical properties of digesta, for which the particle size is likely playing an important role. The aim of the present study was to investigate the effects of WB inclusion and particle size of WB on the microbial composition in the digesta and intestinal mucosa of newly weaned pigs challenged with enterotoxigenic *Escherichia coli* $K88^+$ (ETEC).

5.2. Materials and methods

5.2.1. Animals and diets

The experimental protocol was reviewed and approved by the University of Manitoba Animal Care Committee and pigs were cared for according to the guidelines of the Canadian Council on Animal Care (1993). A total of 36 Genesus ([Yorkshire x Landrace]♀ x Duroc♂) piglets weaned at 17 ± 1 d were obtained from the University of Manitoba's Glenlea Swine Research Unit. The pigs were weighed, individually-housed and randomly assigned to 1 of 4 experimental diets: (1) a negative control diet (NC) based on corn (32%), wheat (20%), barley (17%) and soybean meal (14%); (2) NC + 4% coarsely milled WB (WBc, 1088 μm); (3) NC + 4% finely milled WB (WBf, 445 μm); and (4) a positive control diet (PC) consisting of the NC diet supplemented with a commercial feed grade antibiotic mix (ASP-250: Chlortetracycline, Pencillin G, Sulfamethazine; Alpharma Inc., Fort Lee, NJ). All experimental diets were formulated to meet the NRC (1998) nutrient requirements for piglets weighing 7 to 12 kg (DE, 3400 kcal/kg; CP, 20.9%; Lys, 1.2%). The

animals were housed in a Biohazard Level 2 animal facility that restricted access to unauthorized personal, and all individuals using the facility were trained in procedures related to biohazard containment. Animals had unlimited access to feed and water throughout the 2-week study period, with the room temperature set at 29 ± 1°C.

5.2.2. Experimental procedures and sampling

Animals received the experimental diets from d 1 to d 16 after weaning. On d 9, body weight (BW) and feed intake (FI) were recorded and faecal samples were taken for determination of *E. coli* population and microbial activity. After that, pigs received 6 mL (2.2 x 10^{10} cfu/mL) of a freshly prepared *E. coli* $K88^+$ inoculum following the procedure described by Bhandari et al. (2008). The severity of diarrhoea was assessed using the faecal consistency scoring method of Marquardt et al. (1999). On day 16 after weaning, BW and FI were recorded and animals were euthanized with an intravenous injection of sodium pentobarbitone (50 mg/kg BW). Piglets were bled, and the abdomen was immediately opened to sample ileal digesta and tissue. Segments of the ileum were placed in sterile containers before transportation to the laboratory for microbial analysis. Ileal digesta were divided into two subsamples of about 1 g that were immediately frozen at -80ºC for volatile fatty acid (VFA) and lactic acid determination and for the terminal restriction fragment length polymorphism (T-RFLP) analysis.

5.2.3. Analytical procedures

Dietary dry matter was determined by the standard AOAC (1995) method. Crude protein was quantified by a Leco NS 2000 Nitrogen Analyzer (Leco Corporation, St Joseph, MI). Gross energy was measured with a Parr adiabatic oxygen bomb calorimeter (Parr Instrument Co., Moline, IL). Faecal samples were taken before the experimental infection (d 9) were weighed, diluted and plated on chromogenic *E. coli* media (BBL Levine Eosin Methylene Blue Agar; BD Company, Sparks, USA). Samples from the ileal tissue were also taken in d 16 for microbial assay. A blunt knife was used to scrape the mucosa down to the connective tissue, and the mucosa was then weighed and diluted 10-fold with anaerobic dilution and plated as described previously (Krause et al., 1995). Briefly, 10 µL droplets of

medium were pipetted onto chromogenic *E. coli* media without antibiotic to count the bacterial *E.coli* population and with 0.5 µg/ml of levofloxacin (Fluka, Buchs, Germany) to determine the *E.coli* $K88^+$ serotype adhesion. Dilutions from 10^{-1} to 10^{-9} were plated, allowed to dry before inversion, and incubated at 39ºC for 24 h. The VFA and lactic acid determination were done by gas chromatography as described by Erwin et al. (1961). The extraction of DNA from ileal digesta, as well as the t-RFLP procedure and data analyses were done following the procedure described by Bhandari et al. (2008).

5.2.4. Statistical analyses

Data were subjected to ANOVA, with dietary treatment as the classification factor, using the GLM procedure (SAS Inst., Inc., Cary, NC, USA). Animal was considered as the experimental unit (n = 9). For performance data, initial BW was used as a covariate. The alpha level used for the determination of significance for all the analysis was 0.05 and trends (alpha < 0.10) were also reported.

5.3. Results and Discussion

5.3.1. Piglet performance

Growth performance was not affected by dietary treatments. The average daily feed intake (ADFI) was 231 g and the average daily gain (ADG) was 130 g for the 0 to 16 d period after weaning. The average final BW among treatments was 7.1 kg. These results agree well with those reported by Bhandari et al. (2008) and Wellock et al. (2007) who did not found performance differences within *E. coli* challenged pigs. However, we should remark that the number of animals and experimental conditions were not adequate to obtain clear conclusions from the performance of the animals.

5.3.2. Faecal score and microbiological analysis

The effect of WB on the *E. coli* population in the faeces and in the ileal mucosa, the *E. coli* $K88^+$ serotype count in the ileal mucosa, and the faecal scores (FS) are shown in Table 5.1.

Table 5.1. Effect of wheat bran on the *E. coli* population in the feces (Log_{10} CFU/g digesta) and in the ileal mucosa (Log_{10} CFU/g of tissue) and *E. coli* K88 serotype counts in the mucosa of the ileum (Log_{10} CFU/g of tissue) and the faecal score in early weaned pigs.

Item	Period	Diets[a]				SEM	P-value
		NC	PC	WBf	WBc	(n = 9)	
E. coli population							
Faeces	Day 9	8.4	8.5	8.0	7.4	2.09	0.593
Ileum	Day 16	6.3 ^{x}	6.3 ^{xy}	4.9 ^{y}	4.1 ^{y}	2.11	0.014
E. coli K88 determination							
Ileum	Day 16	4.7 ^{x}	4.7 ^{xy}	2.2 ^{xy}	0.7 ^{y}	2.66	0.021
	6 h	1.5	0.6	1.0	0.5	0.93	0.157
	24 h	1.4	0.6	1.0	0.5	0.75	0.066
Faecal Score[b]	48 h	1.5 ^{x}	0.6 ^{xy}	1.1 ^{xy}	0.5 ^{y}	0.71	0.025
	72 h	1.5 ^{x}	0.5 ^{y}	1.1 ^{xy}	0.5 ^{y}	0.70	0.014
	Overall	1.3 ^{x}	0.5 ^{y}	1.0 ^{xy}	0.5 ^{y}	0.66	0.020

[a]Diets: NC, negative control diet; PC, positive control diet; WBf, wheat bran milled diet and WBc, wheat bran coarse diet.
[b]Faecal score: 0, normal; 1, mild diarrhoea; 2, moderate diarrhoea; 3, severe diarrhoea.
Different superscripts (x and y) in the same row denote significant difference ($P < 0.05$).

Irrespective of the particle size, supplementation of 4% WB in the diet of weaner pigs significantly reduced ($P < 0.05$) *E. coli* population in the ileal mucosa compared with that from pigs fed the NC diet. Furthermore, inclusion of WBc significantly decreased ($P < 0.05$) *E. coli* $K88^{+}$ adhesion to the ileal mucosa compared with that from pigs fed the NC diet. At the same time, FS was lower for piglets fed the WBc and PC diets than those fed a NC diet at 48 h ($P < 0.05$) and 72 h ($P < 0.05$) post-infection. This resulted in a reduction ($P < 0.05$) in the FS for the overall period for those animals receiving the PC and WBc diets. Hermes et al. (2009) also found a reduction in enterobacteria population and faecal score when WBc (4%) and sugar beet pulp (2%) were included in the diets of pigs from week 2 to 5 after weaning. Some reports have suggested that a coarse diet may modify microbiota in the GIT, with reduction in gastric population of enterobacteria, such as Salmonella (Mikkelsen et al., 2004). The authors speculated that processes in the foregut, such as distribution of hydrochloric acid within the stomach content, is favoured when a diet has a coarse structure, and therefore lower numbers of Salmonella reach the small intestine. Canibe et al. (2005) also suggested that

feeding a coarsely ground diet may affect the gastrointestinal ecology of pigs mainly by changing the environment in the proximal GIT. Our result also showed significant differences in the microbial characteristics in the ileal digesta due to WB particle sizes (Table 5.3). While WBc reduced microbial richness in ileal digesta to a similar level as in the antibiotic supplemented diet, WBf increased microbial diversity ($P < 0.001$). It is also interesting to remark that WBf showed a significantly reduced SCFA concentration in ileal digesta than WBc (Table 5.2), which could reflect changes in the foregut digestibility or the transit time of digesta.

Table 5.2. Effect of wheat bran on the faecal (Day 9) and ileal (Day 16) digesta short chain fatty acid (SCFA, mmol/L) concentration in early weaned pigs challenged with ETEC.

Item	Diets[a]				SEM	P-value
	NC	PC	WBf	WBc	(n = 9)	
Faecal SCFA	14.7	14.6	18.2	15.0	5.10	0.276
SCFA profile (%)						
Acetic	61.5	60.7	62.0	59.8	7.03	0.943
Propionic	17.2	18.3	18.4	18.7	3.89	0.842
Butyric	11.0	9.5	10.7	12.7	5.15	0.836
Isobutyric	3.7	3.2	3.0	3.2	0.93	0.568
Valeric	3.3 ^{x}	1.9 ^{xy}	2.8 ^{x}	1.7 ^{y}	0.97	0.006
Isovaleric	3.3	2.6	2.5	2.0	1.04	0.224
Lactic	0.0	0.5	0.4	0.9	1.23	0.708
Ileal SCFA	22.1 ^{xy}	20.0 ^{xy}	14.7 ^{y}	22.4 ^{x}	6.10	0.042
SCFA profile (%)						
Acetic	8.3	12.3	17.2	9.1	8.86	0.129
Propionic	0.6	1.0	1.2	1.0	0.55	0.215
Butyric	0.1	0.2	0.2	0.0	0.28	0.672
Isobutyric	0.0	0.0	0.0	0.1	0.17	0.629
Valeric	-	-	-	-	-	-
Isovaleric	-	-	-	-	-	-
Lactic	91.0	86.5	80.6	89.6	9.66	0.100

[a]Diets: NC, negative control diet; PC, positive control diet; WBf, wheat bran milled diet and WBc, wheat bran coarse diet.
Different superscripts (x and y) in the same row denote significant difference ($P < 0.05$).

Table 5.3. Richness and diversity indices calculated from terminal restriction fragment length polymorphism data of the ileal digesta (collected on d 16 post-weaning) of nursery pigs challenged with ETEC

Diversity index[b]	Diets[a]				SEM	P-value
	NC	PC	WBf	WBc	(n = 9)	
Richness						
Chao2	431.8 ^{x}	195.1 ^{y}	313.0 ^{xy}	230.1 ^{xy}	35.24	0.05
ICE	192.9 ^{x}	133.5 ^{y}	190.2 ^{x}	158.5 ^{xy}	21.29	0.0002
MM mean	237.3 ^{x}	170.6 ^{z}	245.1 ^{x}	188.4 ^{y}	8.80	0.0001
Diversity						
Shannon	1.9 ^{y}	1.9 ^{y}	2.2 ^{x}	2.0 ^{y}	0.09	0.0001
Simpson	2.1 ^{yz}	2.1 ^{z}	2.5 ^{x}	2.2 ^{y}	0.02	0.0001

[a]Diets: NC, negative control diet; PC, positive control diet; WBf, wheat bran milled diet and WBc, wheat bran coarse diet.
Different superscripts (x and y) in the same row denote significant difference ($P < 0.05$).
[b]Diversity indices: Species richness is a statistical estimator of the number of distinct species present, and species diversity is a weighting of the abundance of distinct species. Chao2, the incidence-based coverage estimator (ICE), and the Michaelis-Menten mean (MM mean) are estimators of richness, and the Shannon and Simpson indices are estimators of diversity.

Results of the present study show that incorporation of WB in the diet reduced the ability of *E. coli* and *E. coli* K88 to grow and attach to the intestinal mucosa. The incorporation of WBc also reduced the severity of diarrhoea to values similar to those obtained with the antibiotic-containing diet. It could be hypothesized that greater particle of WB may modify physicochemical properties of digesta, such as the transit time, or the water retention capacity and viscosity of digesta in the small intestine. Such modifications in the physicochemical properties of digesta may have reduced adhesion of *E. coli* to the ileal mucosa and therefore reducing clinical expression of PWC.

5.4. Conclusions

It can be concluded that the incorporation of WB in the diet of early weaned pigs, especially at a coarse particle size, reduces the *E. coli* adhesion in the small intestine and the diarrhoea provoked after an *E. coli* challenge.

5.5. References

AOAC, 1995. In: Association of Official Analytical Chemists (Ed.), Official Methods of Analysis. Arlington, VA, USA.

Awati, A., Williams, B.A., Bosch, M.W., Gerrits, W.J., Verstegen, M.W.A., 2006. Effect of inclusion of fermentable carbohydrates in the diet on fermentation end-product profile in feces of weanling piglets. J. Anim. Sci. 84, 2133-2140.

Bhandari, S.K., Xu, B., Nyachoti, C.M., Giesting, D.W., Krause, D.O., 2008. Evaluation of alternatives to antibiotics using an *Escherichia coli* K88$^+$ model of piglet diarrhea: Effects on gut microbial ecology. J. Anim. Sci. 86, 836-847.

Canadian Council on Animal Care. 1993. Guide to Care and Use of Experimental Animals. 2nd ed. Vol. 1. Can. Counc. Anim. Care. Ottawa, Ontario, Canada.

Canibe, N., Hojberg, O., Hoisgaard, S., Jensen, B.B., 2005. Feed physical form and formic acid addition to the feed affect the gastrointestinal ecology and growth performance of growing pigs. J. Anim. Sci. 73, 1287-1302.

Erwin, E.S., Marco, G.J., Emery, M., 1961. Volatile fatty acids analysis of blood and rumen fluid by gas chromatography. J. Dairy Sci. 44, 1768-1771.

Hampson, D.J. 1994. Postweaning *Escherichia coli* diarrhoea in pigs. Page 171–191 in *Escherichia coli* in Domestic Animals and Humans. C.L. Gyles, ed. CAB International, Wallingford, U.K.

Hermes, R.G., Molist, F., Ywazaki, M., Nofrarías, M., Gómez de Segura, A., Gasa, J., Pérez, J.F., 2009. Effect of dietary level of protein and fiber on the productive performance and health status of piglets. J. Anim. Sci. 87, 3569-3577.

Krause, D.O., Easter, R.A., White, B.A., Mackie, R.I., 1995. Effect of weaning diet on the ecology of adherent lactobacilli in the gastrointestinal tract of the pig. J. Anim. Sci. 73, 2347-2354.

Marquardt, R.R., Jin, L.Z., Kim, J. W., Fang, L., Frohlich, A.A., Baidoo, S.K., 1999. Passive protective effect of egg-yolk antibodies against enterotoxigenic *Escherichia coli* K88$^+$ infection in neonatal and early-weaned piglets. FEMS Immunol. Med. Microbiol. 23, 283-288.

Mikkelsen, L.L., Naughton P.J., Hedemann, M.S., Jensen, B.B., 2004. Effects of physical properties of feed on microbial ecology and survival of *Salmonella*

enterica serovar Typhimurium in the pig gastrointestinal tract. Appl. Environ. Microbiol. 70, 3485-3492.

Molist, F., Gómez de Segura, A., Gasa, J., Hermes, R.G., Manzanilla, E.G., Anguita, M., Pérez, J.F., 2009. Effects of dietary fibre on phsycochemical characteristics of digesta, microbial activity and gut maturation in early weaned piglets. Anim. Feed Sci. Technol. 149, 346-353.

NRC, 1998. Nutrient Requirements of Swine, 10th ed. Natl. Acad. Press, Washington, DC.

Wellock, I.J., Fortomaris, P.D., Houdijk, J.G.M., Wiseman, J., Kyriazakis, I., 2007. The consequences of non-starch polysaccharide solubility and inclusion level on the health and performance of weaned pigs challenged with enterotoxigenic *Escherichia coli*. Br. J. Nutr. 99, 520-530.

CHAPTER 6

"Administration of loperamide and addition of wheat bran to the diets of weaner pigs decrease the incidence of diarrhoea and enhance their gut maturation".

British Journal of Nutrition (Molist et al., 2010a)
Accepted

Abstract

The influence of fibre inclusion and transit time regulation on the performance, health status, microbial activity and population, physicochemical characteristics of the hindgut digesta and intestinal morphology in early weaned pigs were examined. For these experiments, wheat bran (WB) was used as fibre source and loperamide as a drug (LOP) to increase the transit time. In Experiment 1, a total of 128 early weaned pigs were randomly distributed in a 2 x 2 factorial combination of WB inclusion (0 *v*. 40 g/kg) and LOP administration (0 *v*. 0.07 mg/kg body weight) during 13 d. For Experiment 2, a total of twenty-four piglets were allotted to three dietary treatments for 15 d with the same basal diet (control diet) as Experiment 1; a diet with 80g/kg of WB and the combination of WB and LOP. In Experiment 1, LOP improved the average daily feed intake and average daily gain of the animals (P = 0.001 and P = 0.007, respectively). The same result was obtained when WB was combined with LOP. The WB-LOP group also showed a higher concentration of SCFA (P = 0.013), acetic acid (P = 0.004) and propionic acid (P = 0.093). On the other hand, WB inclusion reduced the organic matter and crude protein digestibility (P = 0.001) and tended to decrease the enterobacteria population (P = 0.089). In Experiment 2, WB increased the butyric acid concentration (P = 0.086). We concluded that the inclusion of WB to modify the intestinal microbiota activity combined with LOP may be beneficial to animal health and performance.

6.1. Introduction

Weaning is a critical phase for piglets; it is associated with a variable period of anorexia during the first days after weaning, the deterioration of the digestive function and accumulation of undigested feed as a result of inefficient digestion (Lallès et al., 2007). During this period, piglets are more susceptible to suffer post-weaning diarrhoea, with the proliferation and attachment to the intestinal mucosa of β-haemolytic strains of *Escherichia coli* (Fairbrother et al., 2005). Previous studies have demonstrated that adding sources of dietary fibre in the piglet diets may reduce post-weaning diarrhoea (Molist et al., 2009a).

There is a physiological rationale to support the addition of dietary fibre to young animals. Fermentable carbohydrates constitute the major energy source for microbial fermentation and therefore may act as a link between the piglet and its enteric commensal microbiota (Awati et al., 2006). Adding dietary fibre into the diet can reduce the protein fermentation in the digesta (Hermes et al., 2009), and may normalise the colonic function and the small intestine and colonic mucosa architecture. However, there is conflicting evidence whether NSP promotes a beneficial effect or a detrimental effect on pig health. Thus, some studies have demonstrated that adding sources of mostly insoluble or slowly fermentable NSP (Mateos et al., 2006) or soluble NSP that do not increase viscosity (Wellock et al., 2007) reduce infection-associated symptoms and enhance intestinal structure and function. On the other hand, diets containing soluble NSP sources, which promotes increases in the digesta viscosity, such as pearl barley or guar gum, were associated with increased incidence of enteric disorders (Hopwood et al., 2004).

In earlier studies, we observed that adding wheat bran (WB) in the diet of weaned piglets promoted a beneficial shift in the microbial colonisation of the digestive tract, with a higher production of butyrate in the large intestine and lower enterobacteria counts in the colonic digesta (Molist et al., 2009a) and intestinal mucosa (Molist et al., 2009b). The WB is a source of insoluble NSP that is fairly resistant to microbial degradation in the gastrointestinal tract (GIT) of monogastric animals and reduces the digesta transit time in the small and large bowel (Cummings and Stephen, 1980; Wilfart et al., 2007). We suggested that fermentable carbohydrates from WB were likely influencing bacterial cell growth and activity. However, we were not able to exclude that other changes on the physicochemical properties of digesta or the digesta kinetics might have a role on the changes observed on the intestinal microbial populations. It might be hypothesized that WB might normalise the digestive function and reduce enterobacteria counts by stimulating fermentation and the propulsive digestive motility. In this respect,

butyrate, which is considered the main oxidative fuel for colonocytes, is known to increase with decreased digesta transit time in the GIT (Lewis and Heaton, 2007) or by higher dilution rates in vitro (Oufir et al., 2000).

We designed the present studies to elucidate the role of the digesta transit time in the gut health. To this end we used loperamide (LOP) as a drug to increase digesta transit time. LOP works by decreasing peristalsis and fluid secretion, resulting in longer gastrointestinal transit time and increased absorption of fluids and electrolytes from the GIT (Baker, 2007). It has been extensively used to delay the oro-caecal transit time in human studies (Stephen et al., 1987), in rats (Mittelstadt et al., 2005) and also in pigs (Awouters et al., 1993). With the present study, we aimed to confirm the likely beneficial effects of including WB in the diet of early weaned piglets and to assess which, if any, of the productive, digestive or microbial effects of WB are dominant in changing the digesta transit time.

6.2. Material and methods

6.2.1. Animals and housing

Two experiments were performed at the Animal Facilities of the Universitat Autònoma de Barcelona and received prior approval from the Animal Protocol Review Committee of this institution. The treatment, management, housing, husbandry and slaughtering conditions conformed to the European Union Guidelines (The Council of the European Communities, 1986). In Experiment 1, a total of 128 commercial crossing piglets ((Large White x Landrace) x Pietrain), which had been excluded from receiving creep feed, were weaned at the age of 24d with an average body weight (BW) of 6.4 ± 1.17 kg. Pigs were transported from a commercial farm to the animal facilities and placed into thirty-two pens (4 animals per pen). Each pen had a feeder and a water nipple to ensure *ad libitum* feeding and free water access. The pens were allotted to four treatments (eight replicates for each treatment, Table 6.1) in a 2 × 2 factorial design that included two levels of WB in the diet (0 *v.* 40 g/kg, control diet (CT) *v.* WB, respectively) and two levels of LOP administration (0 or 0.07 mg/Kg BW, named 0 *v.* LOP, respectively). For the Experiment 2, a total of twenty-four piglets of 7.4 ± 1.17 kg from the same origin, breed and age as the previous one were randomly distributed into twelve pens (two animals per pen). The pens were allotted to three treatments (Table 6.1) that included the same basal diet (CT) as Experiment 1; but was modified by adding 8 % of WB and adding WB with LOP (0.07 mg/Kg BW, LOP).

Table 6.1. Diet composition and chemical analysis (g/kg as fed)

Diets[a]	Experiment 1		Experiment 2	
	CT	WB	CT	WB
Raw ingredients (g/kg)				
Corn	332.1	290.3	331.1	280.0
Barley	211.6	210.0	238.2	210.0
Whey powder	130.0	130.0	130.0	130.0
High fat whey	100.0	100.0	100.0	100.0
Soy protein	90.0	90.0	92.5	87.1
Wheat gluten	58.1	55.7	30.0	30.0
Fish meal LT[b]	40.0	40.0	40.0	40.0
Wheat Bran	-	40.0	-	80.0
Sunflower oil	-	6.5	-	6.5
Dicalcium phosphate	10.3	9.6	9.1	8.4
Calcium carbonate	9.3	9.7	11.7	10.6
L-lysine HCL	6.8	6.7	5.2	5.2
DL-Methionine	1.5	1.5	4.1	4.1
L-Threonine	2.1	2.1	2.3	2.3
L-Tryptophan	0.7	0.7	0.6	0.6
Vitamin and mineral premix[c]	4.0	4.0	5.0	5.0
Salt	3.4	3.3	-	-
Chromic oxide	-	-	0.15	0.15
Calculated composition				
ME (MJ/kg)	14.6	14.6	14.4	14.3
Crude protein	208.8	209.1	189.6	190.9
SID lysine	13.75	13.71	12.14	12.12
SID Methionine	4.84	4.79	6.94	6.91
SID threonine	8.72	8.68	8.28	8.23
SID tryptophan	2.67	2.67	2.27	2.29
SID isoleucin	7.77	7.73	7.02	6.96
SID valine	9.07	9.05	8.3	8.26
Chemical analysis				
Dry matter	908.2	907.6	903.0	903.0
GE (MJ/Kg)	17.1	17.5	17.8	17.7
Crude protein (N x 6.25)	200.8	205.6	208.0	210.8
Neutral-detergent fibre	74.0	87.0	85.3	106.3
Acid-detergent fibre	25.0	29.0	18.9	22.9
Diethyl ether extract	67.4	80.5	71.0	75.0
Ash	55.0	57.0	64.0	66.0

[a]Experimental diets: CT, control diet; WB, wheat bran diet.
LT, low temperature; ME, metabolisable energy; CP, crude protein; SID, standardised ileal digestible; GE, gross energy.
[b]Fish meal low temperature: product obtained by removing most of the water and some or all of the oil from fish by heating at low temperature (<70ºC) and pressing.

[c]Supplied per kilogram of feed: 5000 IU of vitamin A, 1000 IU of vitamin D3, 15.0 mg of vitamin E, 1.3 mg of vitamin B1, 3.5 mg of vitamin B2, 1.5 mg of vitamin B6, 0.025 mg of vitamin B12, 10.0 mg of calcium pantothenate, 1.3 g of coline chloride, 15.0 mg of niacin, 15.0 mg of biotin, 0.1 mg of folic acid, 2.0 mg of vitamin K3, 80.0 mg of Fe, 6.0 mg of Cu, 0.7 mg of Co, 60.0 mg of Zn, 30.0 mg of Mn, 0.7 mg of I, 0.1 mg of Se, 0.15 mg of etoxiquin and 1.5 g of chromic oxide.

6.2.2. Experimental procedures and sampling

In Experiment 1, animals received the diets from the first day of the experiment until day 13. LOP (Fortasec®, Esteve, Barcelona, Spain) was administered every morning to the LOP group at a dose of 0.07 mg/Kg BW as an oral solution (0.2 mg/ml LOP). The rest of the animals received the same dose of water. The solutions were carefully administered by a 5 ml plastic syringe fitted with an oesophageal tube. Two experimental periods (0-7 and 7-13 d) were selected to register individual BW, pen feed consumption and piglet health status. On day 10, 0.15 % of chromic oxide was added in the diet to determine the total tract apparent digestibility. On day 12, faeces samples were taken to determine the Cr and SCFA concentrations, and lactobacilli and enterobacteria counts. On day 12, 0.25 % of ferric oxide was also included in the diet to determine the minimum transit time (MTT), which is defined as the time between the administration and the appearance of the red marker in the faeces per pen (Castle and Castle, 1956). For the Experiment 2 all the animals received the experimental diets during 15 d. On day 15, animals were euthanised with an intravenous injection of sodium pentobarbital (200 mg/kg BW). Animals were bled, and the abdomen was immediately opened to tie and remove the whole GIT. Samples from the colon consisted of a pool of all colonic contents. Half of the collected samples were freeze-dried and then dried at 103ºC for complete water removal. The other half were divided into four aliquots: 3 g was collected into previously weighed 10 ml screw cap tubes for water retention capacity (WRC) analysis; the remainder was collected in tubes for water swelling capacity; SCFA; microbial population. Finally, a section of 4 cm from mid-jejunum and 4 cm from the medium colon were removed, opened longitudinally and fixed by immersion in 10 % (v/v) buffered formalin for histological study.

6.2.3. Analytical procedures

Chemical analyses of *the* diets (Table 6.1) were performed according to the Association of Official Analytical Chemists (AOAC, 1995) standard procedures. The chromium oxide concentration in feed and digesta was determined by atomic absorption spectrophotometry following the method of Williams et al. (1962). The WRC of fresh colon digesta contents was determined by centrifugation (2500 *g*/25 min) following Anguita et al. (2007); the water swelling capacity was determined as

the ratio of liquid phase to solid phase obtained after allowing fresh colon digesta to stand for 3 h at room temperature in a test tube. DNA from faeces and colon was extracted and purified using the commercial QIAamp DNA Stool Mini Kit (Qiagen, West Sussex, UK) with some modifications as described by Castillo et al. (2006). Enterobacteria and lactobacilli were quantified by real time PCR using SyBR Green dye following Castillo et al. (2006). The lactobacilli:enterobacteria ratio was calculated by subtracting log 16S rDNA gene lactobacilli copies/g fresh matter (FM) minus log 16S rDNA gene enterobacteria copies/g FM. SCFA and lactic acid concentrations were determined by GC, after submitting the samples to an acid-base treatment followed by diethyl ether extraction and derivatisation, as described by Jensen and Jorgensen (1994). Tissue samples for the histological study were dehydrated and embedded in paraffin wax, sectioned at 4 µm and stained with haematoxylin and eosin. Morphometric measurements were performed with a light microscope (BHS, Olympus, Spain). Villus height and crypt depth, and the globet cell number in crypts were measured. Measurements were taken in ten well-oriented villi and crypts from each intestinal section of each animal. The villus height and crypt depth were measured using a linear ocular micrometer (Olympus, Ref. 209-35 040; Microplanet, Barcelona, Spain). On the basis of the cellular morphology, differences between globet cells and lymphocytes were clearly distinguishable at 400 x magnification. Cell density was expressed as the number of lymphocytes per 1000 μm^2. All morphometric analyses were done by the same person, who was blind to the treatments.

6.2.4. Statistical analyses

In Experiment 1 results *on* productive performance, microbial counts, organic matter (OM) and crude protein (CP) digestibility, MTT and SCFA in the faeces were subjected to ANOVA using the GLM procedure (SAS Inst., Inc., Cary, NC, USA). Data were analysed as a 2 x 2 factorial arrangement of treatments, with diet and LOP treatment as the factors in four randomised blocks. Productive performance data were adjusted for initial live weight by covariance analysis. In Experiment 2, results on OM and starch digestibility, physicochemical characteristics, SCFA and lactic acid and microbial population of the colonic digesta and morphometry of the intestinal mucosa were subjected to ANOVA with diet as the classification factor, using the GLM procedure (SAS Inst., Inc., Cary, NC, USA). In both the experiments means presented in the tables are least square means, the pen was considered as the experimental unit. Differences were considered significant at $P < 0.05$. Tendencies for $0.05 < P < 0.15$ were also presented.

6.3. Results

6.3.1. Experiment 1

6.3.1.1. Animal performance, health status and nutrient digestibility

Data on feed intake and growth performance are shown in Table 6.2. The pigs receiving the LOP treatments showed a higher average daily feed intake (P = 0.001) than animals without it. Differences were more pronounced in animals fed on the WB diet, reflecting the tendency in the interaction with WB and LOP during the second week (P = 0.070) and the overall period (P = 0.069). A significant effect of the experimental treatments was also observed for the average daily gain (ADG) of the animals during weeks 1 and 2 after weaning. LOP and WB increased the ADG of the animal, with LOP pigs fed on the WB diet showing a much larger increase in ADG than the rest of experimental treatments (interaction between WB and LOP, P = 0.047). As a result of differences on the average daily feed intake and the ADG, pigs fed on the WB diet increased the feed efficiency (P = 0.013) during the 2-week period, while LOP increased feed efficiency during week 1 (P = 0.005).

Table 6.2. Body weight (BW), average daily feed intake (ADFI), average daily gain (ADG) and gain : feed ratio (G:F) in early weaned pigs (Experiment 1)

Item	Diets[a]				SEM (n = 8)	P-diet[b]		
	CT		WB					
	0	LOP	0	LOP		DIET	LOP	D x L
Body Weight (g)								
Initial	6430	6400	6390	6390	11.4	0.952	0.970	0.969
Final	8590	8850	8610	9630	12.8	0.382	0.168	0.408
ADFI (g/animal and day)								
Week 1	189	221	183	248	33.3	0.408	0.001	0.210
Week 2	354	382	328	424	50.6	0.660	0.001	0.070
Overall	271	293	253	325	37.5	0.610	0.001	0.069
ADG (g/animal and day)								
Week 1	82	127	95	191	40.0	0.017	0.001	0.101
Week 2	234	248	233	327	71.3	0.131	0.050	0.129
Overall	157 [y]	170 [y]	156 [y]	238 [x]	47.0	0.050	0.007	0.047
G : F								
Week 1	0.43	0.58	0.53	0.77	0.171	0.0328	0.0052	0.4540
Week 2	0.66	0.63	0.70	0.77	0.147	0.0954	0.6777	0.3658
Overall	0.57	0.57	0.62	0.77	0.115	0.0135	0.1728	0.1737

Different superscripts (x, y) in the same row denote significant differences (P < 0.05)

[a]Diets: CT, control diet; WB, wheat bran diet; LOP, loperamide.

[b]P-diet: DIET, effect inclusion CT or WB in diet; D x L, effect diet and loperamide treatment.

Table 6.3 shows the number of pigs with diarrhoea and the mortality rate, as well as the total tract apparent digestibility of OM and CP. The LOP treatment reduced the number of pigs suffering diarrhoea (P = 0.029). This effect was essentially observed with the WB diet (tendency for an interaction, P = 0.096). However, no significant differences in the mortality were observed between treatments. The incorporation of WB in the diet reduced the total tract digestibility of OM (P = 0.001) and CP (P = 0.001). On the other hand, LOP tended to improve the coefficient of OM (P = 0.061) and improved the CP (P = 0.026) digestibility, especially with the WB diets (P interaction = 0.074 and 0.116 for CP and OM digestibility, respectively). No significant differences among diets were observed in the MTT registered on day 13 after weaning, averaging 13.7, 15.5, 13.4 and 14.4 hours for the CT-0, CT-LOP, WB-0 and WB-LOP treatments, respectively. However, LOP tended (P = 0.070) to increase the MTT compared to the non-treated animals.

Table 6.3. Mortality, pigs with diarrhoea per treatment and coefficient of total tract apparent organic matter and crude protein digestibility in early weaned pigs (Experiment 1)

Item	Diets[a]				SEM	P-diet[b]		
	CT		WB		(n = 8)			
	0	LOP	0	LOP		DIET	LOP	D x L
Animal health status (nº of pigs)								
Mortality	2/32	2/32	2/32	1/32	0.44	0.690	0.690	0.690
Diarrhoea	9/30	8/30	13/31	4/32	0.24	0.969	0.029	0.096
Total tract apparent digestibility								
Organic Matter	83.2	83.7	72.0	78.0	6.75	0.001	0.061	0.116
Crude Protein	83.8	84.6	68.7	76.0	6.96	0.001	0.026	0.074

Different superscripts (x, y) in the same row denote significant differences (P < 0.05)
[a]Diets: CT, control diet; WB, wheat bran diet; LOP, loperamide.
[b]P-diet: DIET, effect inclusion CT or WB in diet; D x L, effect diet and loperamide treatment.

6.3.1.2. Fermentation end-products and quantitative changes in the microbial population of faeces

Total SCFA concentration and microbial counts in faeces are shown in Table 6.4.

Table 6.4. Concentration of SCFA and bacterial population (enterobacteria and lactobacilli) on faeces of piglets, 13 d after weaning (Experiment 1)

Item	Diets[a]				SEM	P-diet[b]		
	CT		WB		(n = 8)			
	0	LOP	0	LOP		DIET	LOP	D x L
Concentration (µmol/g FM) of SCFA								
Total SCFA	103.0 ^{xy}	102.2 ^{xy}	90.9 ^{y}	114.8 ^{x}	18.35	0.642	0.021	0.013
Acetic	66.1 ^{xy}	63.3 ^{xy}	57.1 ^{y}	73.3 ^{x}	11.85	0.641	0.037	0.004
Propionic	22.2	22.1	19.9	23.5	4.106	0.454	0.109	0.093
Butyric	8.9	10.5	8.8	11.7	3.147	0.625	0.009	0.401
Isoacids	3.5	3.9	2.8	3.1	1.436	0.062	0.384	0.824
Branched chain ratio	0.035	0.041	0.034	0.031	0.0142	0.1891	0.7688	0.2634
Bacterial population measured by Real-Time PCR (log 16S rDNA gene copies /g FM)								
Enterobacteria	9.0	9.3	8.8	8.5	0.68	0.089	0.965	0.295
Lactobacilli	9.6	9.4	9.4	9.1	0.46	0.291	0.216	0.818

Different superscripts (x, y) in the same row denote significant differences ($P < 0.05$)
[a]Diets: CT, control diet; WB, wheat bran diet; LOP, loperamide.
[b]P-diet: DIET, effect inclusion CT or WB in diet; D x L, effect diet and loperamide treatment.

A significant interaction was observed between WB and LOP groups (P = 0.013) on the total SCFA concentration. The LOP administration increased the faecal SCFA concentration in piglets fed on the WB diet, whereas no differences were observed in the CT group. LOP treatment increased the concentration of acetic acid (P = 0.004) and tended to increase the propionic acid (P = 0.093) in piglets fed on the WB diet and increased (P = 0.009) the butyric acid in both the groups of animals (CT and WB). On the other hand, piglets fed on the WB diets tended to reduce (P = 0.062) concentration of isoacids. The piglets fed on the WB diet tended (P = 0.089) also to show lower counts of enterobacteria in the faeces compared with the CT diet. No significant differences were observed in the lactobacilli counts or associated with the LOP treatment.

6.3.2. Experiment 2

6.3.2.1. Digestion and morphometry of the intestinal mucosa

LOP increased (P = 0.010) the digestibility of OM (data not shown). LOP also promoted significant changes in the morphometry of the jejunum, with an increase (P = 0.031) in the villus height:crypt depth ratio compared with the WB diet (data not shown). No other significant differences were observed in any of the variables studied in the jejunum. In the colon no significant differences were observed among the dietary treatments in the morphometric and the cellular measurements.

6.3.2.2. Physicochemical characteristics, fermentation parameters and microbial population of the colonic digesta

Data related to the physicochemical characteristics of digesta (WRC and unbound water) are presented in Fig. 6.1. Feeding animals with WB diet and LOP administration increased (P = 0.001) the WRC compared with the CT diet. On the other hand, animals fed on the WB diet showed the lowest concentration (P = 0.018) of unbound water compared with the CT diet. No significant differences between treatments were observed on the total concentration of SCFA and the concentration of acetic, propionic, isoacids and lactic acid (data not shown). On the other hand, the butyric acid concentration tended (P = 0.086) to be higher in pigs fed on the WB diet (35.9 and 20.3 μmol/g FM for the WB and the WB-LOP groups, respectively) compared with the CT diet (11.7 μmol/g FM). No significant differences were observed on the enterobacteria and lactobacilli counts (data not shown).

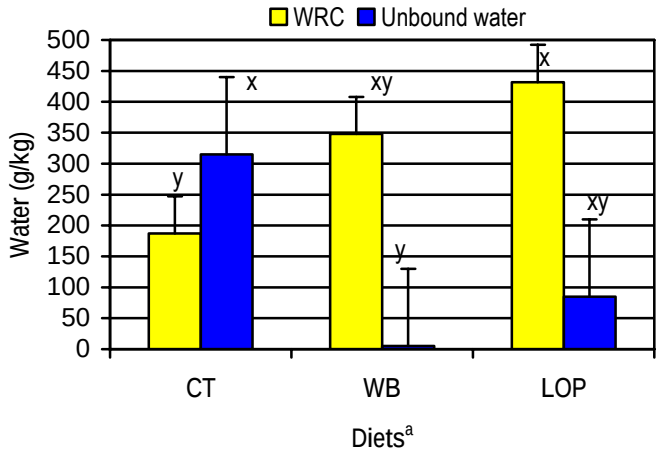


Fig. 6.1. Water retention capacity and unbound water of colonic digesta on piglets fed experimental diets. [a]Diets: CT, control diet; WB, wheat bran diet; LOP, animals treat with loperamide. Values are least square means and standard error of the mean (n=4). Different superscripts (x and y) denote significant difference between diets ($P < 0.05$). The P-value for diets were 0.01 and 0.018 for the WRC and the unbound water respectively (Experiment 2).

6.4. Discussion

6.4.1. The influence of wheat bran on the adaptation of piglets after weaning

Dietary fibre has become one of the dietary components, which has attracted much interest in connection with the nutrition of young animals. Previous studies have demonstrated that adding sources of mostly insoluble low-fermentable NSP (such as oat bran) to the diets for weaned pigs can ameliorate the incidence of diarrhoea and the animal performance (Mateos et al., 2006; Kim et al., 2007). The basis for this protective effect is still uncertain, but the authors suggested that it could be related to changes in the numbers and metabolic activity of selected components of the intestinal microbiota.

The WB (the coarse outer membrane of the wheat kernel) was chosen for the present study because of its high proportion of NSP as insoluble cellulose and arabinoxylans (Bach Knudsen, 1997) and its large particle size. Our results show that pigs fed on the WB treatment showed a reduction in the total tract digestibility of the OM and CP, but underwent an increase in the feed efficiency. Numerous reports

indicate that dietary fibre reduces the total tract digestibility of protein and energy. In practice, fibrous diet components dilute the nutrient in feed because the NSP fraction is digested to a lower extent than other fractions, such as those of starch, CP or fat (Morales et al., 2002; Bach Knudsen et al., 2005). Moreover, changes in the physical characteristics of the intestinal contents due to the presence of specific fibre components may increase the viscosity, influence gastric emptying or slow the diffusion or mobility of enzymes, substrates and nutrients to the absorptive surface. The consequence is that fibre may reduce nutrient digestibility of fat (Freire et al., 2000) or increase the endogenous nitrogen excretion (Schulze et al., 1995). This effect was quantitatively described by Le Goff et al. (2002). They found that the impact of the neutral-detergent fibre fraction on the digestibility coefficient of energy is significant, with approximately 0.1 % reduction per 1 g neutral-detergent fibre/kg DM.

In the literature, it is also generally accepted that fibre in the growing pig diet may also reduce the voluntary intake and the BW gain of the animals. In the present study, the pigs fed on the WB diets did not lower their voluntary intake and even increased the gain:feed efficiency. Some authors (Kyriazakis et al., 1995; Mateos et al., 2006 ; Molist et al., 2009a) have reported that moderate levels of WB or oats hulls in post-weaning diets increased the feed consumption of pigs. The authors suggested that young piglets may have a minimum requirement of fibre for correct functioning of the digestive tract. However, we should not exclude the possibility that the increased weight gain efficiency observed in the present study could be due, at least in part, to the increased weight of the internal organs, possibly by a higher weight of the gut contents (Pond et al., 1986).

Including WB in the diet reduced the branched-chain fatty acid concentration and tended to decrease the enterobacteria population in the faeces. Previous results from our group have also indicated that incorporation of WB to the diet also decreased the enterobacteria counts in the caecum digesta (Molist et al., 2009a) and the K88 *E. coli* attachment to the ileum mucosa after an experimental infection (Molist et al., 2009b). Although enterobacteria contain numerous species of bacteria, its reduction may indicate a beneficial shift in the composition of the microbial population. In this respect, different authors have demonstrated that the

inclusion of fermentable carbohydrates in weanling diets may reduce the protein fermentation along the GIT (Awati et al., 2006; Bikker et al., 2006) being related with the reduction in isoacids observed here. Protein fermentation in the digestive tract is considered as a potential risk for dysbiosis and proliferation of pathogenic bacteria (Prohaszka and Baron, 1980). Pigs fed on the WB diet tended also to show a higher concentration of butyric acid in the colon. In this respect, butyrate is considered an important metabolite because it is the principal oxidative fuel for the colonocytes and may have beneficial trophic effects on the inflamed caeco-colonic mucosa (Oufir et al., 2000). It is accepted that starch and bran from wheat or oat stimulate the formation of butyrate (Bugaut, 1987), while xylans and pectin rich fractions are all associated with a related low formation of butyrate (Hughes et al., 2007).

On the other hand, fibre is also able to modify the physicochemical properties of digesta. WB, due to its high content of insoluble fibre, is known to improve constipation in human subjects (Cann et al., 1984) and reduces the mean retention time of digesta in the small intestine of pigs (Wilfart et al., 2007). In the present study, we were not able to detect differences in the MTT with the WB supplementation, but we observed a significant increase in the WRC and a reduced percentage of unbound water in the colonic digesta. The results demonstrate the higher water-binding capacity of the insoluble long-chain NSP as compared with other compounds, such as starch or protein (Anguita et al., 2007). Moreover, these changes could suggest that the physicochemical properties of digesta could have a role in some GIT processes, such as the gastric emptying, the small intestine motility or the hindgut fermentation. Some reports have suggested that a coarse diet may modify the physicochemical and microbial properties of digesta contents, with decreases in the survival level of some enterobacteria, such as *Salmonella* (Mikkelsen et al., 2004). The authors speculated that processes in the foregut, such as distribution of HCl within the stomach content, is favoured when a diet has a coarse structure and a higher WRC, so that lower counts of Salmonella reach the small intestine.

6.4.2. The influence of loperamide on the adaptation of piglets to the diet

LOP is a synthetic opiate derivative frequently used as antidiarrhoeal drug in human subjects. It decreases the motility of the circular and longitudinal smooth muscles of the intestinal wall, slows down the flow entering the colon and stimulates

colonic water absorption (Schiller et al., 1984). While LOP is widely used in adults, there has been concern about the safety of using this drug for young children and during the course of infectious diarrhoea. The contraindications in cases of invasive bacterial infections come from the risk of aggravating the symptoms per digesta stasis allowing bacterial translocation. Results from the present study indicate that LOP tended to increase the MTT along the GIT and increased the total tract and foregut digestibility of OM and CP, especially with the WB diet. These results were associated with a significant increase in the villus height:crypt depth ratio in the jejunum (Experiment 2), which is considered a useful criterion for estimating the digestive capacity in the small intestine (Montagne et al., 2003). In contrast, other authors have reported that LOP strongly inhibits pancreaticobiliary secretion (bilirubin and amylase), acting on the nerve supply to the pancreas and gallbladder (Appia et al., 1984; Thimister et al., 1997).

Treating animals with LOP also increased the average daily feed intake and the ADG during the first 2 weeks after weaning. LOP is known to affect not only the opioid receptors related to inhibition of intestinal motility, but also those related to analgesia that are present on the peripheral sensory nerves and is up-regulated during the development of inflammation (Stein et al., 2001). After weaning, the stresses that the animals suffer lead to a period of anorexia that may contribute to a local inflammation in their small intestine (McCracken et al., 1999). Moreover, the reduced enteral feeding during the first days after weaning is considered to be the cause of the impairment of the piglet gut barrier and the shortened villi. It appears that treating animals with LOP early after weaning may reduce the intestinal inflammation and improve the behaviour of the animal; this resulted in a higher feed consumption, weight gain and improved mucosa integrity. This is in good agreement with Bowden et al. (1987), who reported positive effects of anti-inflammatory analgesic drugs and muscarinic receptor blocking agents on appetite in the pig.

The increase in the digestibility of WB diets with LOP was also associated with a significant increase in the concentration of SCFA, acetic, propionic and butyric acid in the faeces, which likely result from an advanced maturation of the colonic digestive tract. Graham et al. (1986) and Le Goff et al. (2002) suggested that the fibre degrading capacity in the pig intestine increases with age, likely due to increases on the transit time and the metabolic activity of the microbiota.

6.5. Conclusions

The inclusion of a moderate level of an insoluble fibre ingredient such as WB that could modify the intestinal microbiota activity, together with a drug like LOP, that has effects on the intestinal motility and peripheral analgesia, to the post-weaning diet, may have beneficial effects with regard to animal health and performance.

6.6. Acknowledgments

This research w*as supported* by the Spanish CICYT (project AGL2005-07438-C02-01). We thank the Ministerio de Educación, Cultura y Deporte, Spain for research fellowships. We also thank the Servei de Granges i Camps Experimentals de la UAB for its service and assistance during the experiment. There are no conflicts of interest statements between the authors. F.M.G., M.Y., A.G. de S. U., R. G. H., J. F. P. H. have participated in the research and in the writing of the manuscript. J. G. G. has participated in the writing of the manuscript.

6.7. References

Anguita, M., Gasa, J., Nofrarias, M., Martín-Orúe, S.M., Pérez, J.F., 2007. Effect of coarse ground corn, sugar beet pulp and wheat bran on the voluntary intake and physicochemical characteristics of digesta of growing pigs. Livest. Sci. 107, 182-191.

AOAC, 1995. In: Association of Official Analytical Chemists (Ed.), Official Methods of Analysis. Arlington, VA, USA.

Appia, F., Chariot, J., Roze, C., Delatour, J., Vaille, C., 1984. Loperamide induced inhibition of pancreatic secretion in rats. Eur. J. Pharmacol. 103, 71-79

Awati, A., Williams, B.A., Bosch, M.W., Gerrits, W.J.J., Verstegen, M.W.A., 2006. Effect of inclusion of fermentable carbohydrates in the diet on fermentation end-product profile in feces of weanling piglets. J. Anim. Sci. 84, 2133-2140.

Awouters, F., Megens, A., Verlinden, M., Schuurkes, J., Niemegeers, C., Janssen, P.A.J., 1993. Loperamide - survey of studies on mechanism of its antidiarrheal activity. Dig. Dis. Sci. 38, 977-995.

Bach Knudsen, K.E., 1997. Carbohydrate and lignin contents of plant materials used in animal feeding. Anim. Feed Sci. Technol. 67, 319-338.

Bach Knudsen, K.E., Serena, A., Kjaer, A.K.B., Jorgensen, H., Engberg, R., 2005. Rye bread enhances the production and plasma concentration of butyrate but not the plasma concentrations of glucose and insulin in pigs. J. Nutr. 135, 1696-1704.

Baker, D.E., 2007. Loperamide: a pharmacological review. Rev. Gastr. Dis. 7, S11-S18.

Bikker, P., Dirkzwager, A., Fledderus, J., Trevisi. P., Le Huërou-Luron, I., Lallès, J.P., Awati, A., 2006. The effect of dietary protein and fermentable carbohydrates levels on growth performance and intestinal characteristics in newly weaned piglets. J. Anim. Sci. 84, 3337-3345.

Bowden, H.C., Sharman, D.F., Stephens, D.B., 1987. Effects of antiinflammatory analgesic drugs and muscarinic receptor blocking agents on appetite in the pig. J. Vet. Pharmacol. Ther. 10, 269-272.

Bugaut, M., 1987. Occurrence, absorption and metabolism of short chain fatty acids in the digestive tract of mammals. Comp. Bioch. Physiol. 86, 439-472.

Cann, P.A., Read, N.W., Holdsworth, C.D., 1984. What is the benefit of coarse wheat bran in patients with irritable bowel syndrome. Gut. 25, 168-173.

Castillo, M., Martín-Orúe, S.M., Roca, M., Manzanilla, E.G., Badiola, I., Pérez, J.F, Gasa, J., 2006. The response of gastrointestinal microbiota to avilamycin, butyrate, and plant extracts in early-weaned pigs. J. Anim. Sci. 84, 2725-2734.

Castle, E.J., Castle, M.E., 1956. The rate of passage of food through the alimentary tract of pigs. J. Agric. Sci. 47, 196-203.

Cummings, J.H., Stephen, A.M., 1980. The role of dietary fiber in the human-colon. Can. Med. Assoc. J. 123, 1109-1114.

Fairbrother, J.M., Nadeau, E., Gyles, C.L., 2005. *Escherichia coli* in postweaning diarrhea in pigs: an update on bacterial types, pathogenesis, and prevention strategies. Anim. Health Res. Rev. 6, 17-39.

Freire, J.P.B., Guerreiro, A.J.G., Cunha, L.F., Aumaitre, A., 2000. Effect of dietary fibre source on total tract digestibility, caecum volatile fatty acids and digestive transit time in the weaned piglet. Anim. Feed Sci. Technol. 87, 71-83.

Graham, H., Hesselman, K., Aman, P., 1986. The influence of wheat bran and sugar-beet pulp on the digestibility of dietary components in a cereal based pig diet. J. Nutr. 116, 242-251.

Hermes, R.G., Molist, F., Ywazaki, M., Nofrarias, M., Gómez de Segura, A., Gasa, J., Pérez, J.F., 2009. Effect of dietary level of proteína and fiber on the productive performance and health status of piglets. J. Anim. Sci. 87, 3569-3577.

Hopwood, D.E., Pethick, D.W., Pluske, J.R., Hampson, D.J., 2004. Addition of pearl barley to a rice-based diet for newly weaned piglets increases the viscosity of the intestinal contents, reduces starch digestibility and exacerbates post-weaning colibacillosis. Br. J. Nutr. 92, 419-427.

Hughes, S.A., Shewry, P.R., Li, L., Gibson, G.R., Sanz, M.L., Rastall, R.A., 2007. *In vitro* fermentation by human fecal microflora of wheat arabinoxylans. J. Agric. Food Chem. 55, 4589-4595.

Jensen, B.B., Jorgensen, H., 1994. Effect of dietary fiber on microbial activity and microbial gas production in various regions of the gastrointestinal tract of pigs. Appl. Environ. Microbiol. 60, 1897-1904.

Kim, J.C., Mullan, B.P., Hampson, D.J., Pluske, J.R., 2007. Addition of oat hulls to an extruded rice-based diet for weaner pigs ameliorates the incidence of diarrhoea and reduces indices of protein fermentation in the gastrointestinal tract. Br. J. Nutr. 99, 1217-1225.

Kyriazakis, I., Emmans, G.C., Anderson, D.H., 1995. Do breeds of pig differ in the efficiency with which they use a limiting protein supply?. Br. J. Nutr. 74, 183-195.

Lallès, J.P., Bosi, P., Smidt, H., Stokes, C.R., 2007. Weaning – a challenge to gut physiologists. Livest. Sci. 108, 82-93.

Le Goff, G., van Milgen, J., Noblet, J., 2002. Influence of dietary fibre on digestive utilization and rate of passage in growing pigs, finishing pigs and adult sows. Anim. Sci. 74, 503-515.

Lewis, S.J., Heaton, K.W., 1997. Increasing butyrate concentration in the distal colon by accelerating intestinal transit. Gut. 41(2), 245-51.

Mateos, G.G., Martín, F., Latorre, M.A., Vicente, B., Lazaro, R., 2006. Inclusion of oat hulls in diets for young pigs based on cooked maize or cooked rice. Anim. Sci. 82, 57-63.

McCracken, B.A., Spurlock, M.E., Roos, M.A., Zuckermann, F.A., Gaskins, H.R., 1999. Weaning anorexia may contribute to local inflammation in the piglet small intestine. J. Nutr. 129, 613-619.

Mikkelsen, L.L., Naughton, P.J., Hedemann, M.S., Jensen, B.B., 2004. Effects of physical properties of feed on microbial ecology and survival of *Salmonella* enterica serovar Typhimurium in the pig gastrointestinal tract. Appl. Environ. Microbiol. 70, 3485-3492.

Mittelstadt, S., Hemenway, C., Spruell, R., 2005. Effects of fasting on evaluation of gastrointestinal transit with charcoal meal. J. Pharmacol. Toxicol. Meth. 52, 154-158.

Molist, F., Gómez de Segura, A., Gasa, J., Hermes, R.G., Manzanilla, E.G., Anguita, M., Pérez, J.F., 2009a. Effects of the insoluble and soluble dietary fibre on the physicochemical properties of digesta and the microbial activity in early weaned piglets. Anim. Feed Sci. Technol. 149, 346-53.

Molist, F., Gómez de Segura, A., Perez, J.F., Bhandari, S.K., Krause, D.O., Nyachoti, C.M., 2009b. Effect of wheat bran on the health and performance of weaned pigs challenged with *Escherichia coli* K88$^+$. Livest. Sci. (In the Press).

Montagne, L., Pluske, J.R., Hampson, D.J., 2003. A review of interactions between dietary fibre and the intestinal mucosa, and their consequences on digestive health in young non-ruminant animals. Anim. Feed Sci. Technol. 108, 95-117.

Morales, J., Perez, J.F., Baucells, M.D., Mourot, J., Gasa, J., 2002. Comparative digestibility and lipogenic activity in Landrace and Iberian finishing pigs fed ad libitum corn- and corn-sorghum-acorn-based diets. Livest. Prod. Sci. 77, 2-3.

Oufir, L.E., Barry, J.L., Flourie, B., Cherbut, C., Cloarec, D., Bornet, F., Galmiche, J.P., 2000. Relationships between transit time in man and in vitro fermentation of dietary fiber by fecal bacteria. Eur. J. Clin. Nutr. 54, 603-609.

Pond, W.G., Pond, K.R., Ellis, W.C., Matis, J.H., 1986. Markers for estimating digesta flow in pigs and the effects of dietary fiber. J. Anim. Sci. 63, 1140-1149.

Prohaszka, L., Baron, F., 1980. The predisposing role of high dietary protein supplies in enteropathogenic *Escherichia coli* infections of weaned pigs. Zentralbl Veterinarmed B 27, 222-232.

Schiller, L.R., Ana, C.A.S., Morawski, S.G., Fordtran, J.S., 1984. Mechanism of the antidiarrheal effect of loperamide. Gastroenterol. 86, 1475-1480.

Schulze, H., Vanleeuwen, P., Verstegen, M.W.A., Vandenberg, J.W.O., 1995. Dietary level and source of neutral detergent fiber and ileal endogenous nitrogen flow in pigs. J. Anim. Sci. 73, 441-448.

Stein, C., Machelska, H., Schafer, A., 2001. Peripheral analgesic and antiinflammatory effects of opioids. Zeitschrift. Rheumatol. 60, 416-424.

Stephen, A.M., Wiggins, H.S., Cummings, J.H., 1987. Effect of changing transit-time on colonic microbial-metabolism in man. Gut 28, 601-609.

The Council of the European Communities, 1986. Council Directive 86/609/EEC of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of Animals used for Experimental and Scientific purposes. Available: http://europa.eu.int/comm/environment/chemicals/lab_animals/legislation_en.htm Accessed Apr. 25, 2006.

Thimister, P.W.L., Hopman, W.P.M., van Roermund, R.F.C., Willems, H.L., Rosenbusch, G., Woestenborghs, R., Jansen, J.B.M.J., 1997. Inhibition of pancreaticobiliary secretion by loperamide in humans. Hepatology 26, 256-261.

Wellock, I.J., Houdijk, J.G.M., Kyriazakis, I., 2007. Effect of dietary non-starch polysacharide solubility and inclusion level on gut health and the risk of post weaning enteric disorders in newly weaned piglets. Livest. Sci. 108, 186-189.

Wilfart, A., Montagne, L., Simmins, H., Noblet, J., van Milgen, J., 2007. Digesta transit in different segments of the gastrointestinal tract of pigs as affected by insoluble fibre supplied by wheat bran. Br. J. Nutr. 98, 54-62.

Williams, C.H., Iismaa, O., David, D.J., 1962. Determination of chromic oxide in faeces samples by atomic absorption spectrophotometry. J. Agric. Sci. 59, 381-385.

CHAPTER 7

"The interaction between wheat bran and pharmacological doses of zinc oxide may reduce their effects on the intestinal microbiota of early weaning piglets".

British Journal of Nutrition (Molist et al., 2010b)
Under revision

Abstract

Three experiments were designed to evaluate: 1.- the ability of wheat bran (WB) to bind *E. coli* (Experiment 1). 2.- effects of including WB and ZnO in the diet of early weaning piglets on the performance and microbial activity in the GIT (Experiment 2); 3.- the interactions between WB and ZnO regarding *E. coli* growth (Experiment 3). In Trial 2, 64 piglets were distributed in a 2 x 2 factorial combination of two levels of WB (0 *v*. 40 g/kg) and ZnO (0 *v*. 3 g/kg) in the diet. In Experiment 3, a 4 x 2 factorial design with four different solutions combining WB, phytase or xylanase, and the two levels of ZnO were tested for their ability to modify *E. coli* growth. Experiment 1 showed that *E. coli* K88 adhered more strongly to WB than to other fibre sources. In Experiment 2, inclusion of ZnO in the diet improved the growth and gut health but reduced hindgut fermentation. Inclusion of WB increased SCFA concentrations, and decreased *E. coli* counts. Simultaneous incorporation of WB and ZnO increased *E. coli*. Experiment 3 confirmed the interaction between the WB phytates and ZnO, as reflected in the lower antimicrobial activity of the WB-ZnO compared to the WB-phytase-ZnO and ZnO. We can conclude that incorporation of WB in the diet improved gut health by modulating the activity of the microbiota and blocking the adhesion of *E. coli* to the intestine. The negative interaction between WB and ZnO make necessary to consider inclusion of phytase enzymes in diets.

7.1. Introduction

Post-weaning colibacillosis diarrhoea is considered a major problem during the nursery period of the growing pig. The main aetiological agents are different strains of *E. coli* (ETEC)(Hampson, 1994) especially those expressing the fimbrial antigens which mediate the adhesion to specific receptors on the brush borders of villous enterocytes (Bertschinger et al., 1972; Francis et al., 1998). So far, the most common strategy to prevent the proliferation of pathogenic bacteria in the intestine has been the addition of in-feed antimicrobial agents in the post-weaning diets (Verstegen and Williams, 2002) (ie. therapeutic doses of zinc oxide (ZnO) are extensively used in the pig industry to reduce the incidence of post-weaning *E. coli* diarrhoea (Cardinal et al., 2006)). However, the risk of generating new microbial resistance and environmental concerns about the excessive excretion of some minerals in the faeces has resulted in growing restrictions on its use in the European Union.

Alternatively, some studies have shown that an adequate selection of the main ingredients in the diet can significantly improve the piglet digestive adaptation after weaning. It is accepted that high amounts of crude protein (CP) in the diet of newly weaned piglets may predispose them to post-weaning colibacillosis because of the high buffering capacity of dietary protein in the stomach and the higher protein fermentation in the gastrointestinal tract (GIT) (Ball and Aherne, 1987). In contrast, different reports support the hypothesis that low-CP diets (Nyachoti et al., 2006; Heo et al., 2009), protein of animal origin (Cardinal et al., 2006) or diets supplemented with fermentable carbohydrates (high lactose levels (Pierce et al., 2007); or wheat bran and sugar beet pulp (Bikker et al., 2006; Hermes et al., 2009)) help to maintain the enteric health by lowering the protein fermentation. Moreover, it is considered that the inclusion of fermentable low viscous carbohydrates can modulate the gastrointestinal microbiota by increasing the growth of lactic acid bacteria (Houdijk et al., 2002) which, in some way, exhibit prebiotic functions. On the other hand, there are reports which suggest that moderate levels of low-fermentable insoluble fibre sources (barley hulls (Hedemann et al., 2006) and oat hulls (Mateos et al., 2006)) may also improve gut morphology and reduce the incidence of diarrhoea. Previous results from our group showed that the inclusion of wheat bran (WB) in the

diet of early weaned piglets diminished the incidence of diarrhoea and the attachment of *E. coli* K88 to the ileum mucosa after an experimental infection (Molist et al., 2009b). The incorporation of WB in the diet decreased the enterobacteria and coliform counts and increased the water retention capacity (WRC) and the butyrate concentration (Molist et al., 2009b; 2010a). We suggested that an increase of fermentation and likely changes in the physicochemical properties of digesta could be involved in the inhibitory effect of WB on the growth of opportunistic pathogens. Additionally, other more specific mechanisms could also be involved. In this regard recent works have shown, *in-vitro* (Becker and Galleti, 2008) and *in-vivo* (Becker et al., 2009), that dietary fibres from plants, because of their carbohydrate nature and low digestibility, may act as receptor analogues which could block the attachment of *E. coli* to the intestinal tract. It is well known, that intestinal pathogens to effectively colonize a host animal and cause disease have developed means for attachment or adhesion to the host cells and tissues (Ofek et al., 2003). Means of bacterial adhesion mainly involve surface lectins that combine with complementary carbohydrates present on the host cell surface (Sharon and Lis, 1989). Blocking or inhibiting these lectins by suitable carbohydrates or their analogues has been suggested as a strategy to prevent and treat some microbial diseases, such as diarrhoea in pigs caused by the *E. coli* K88 (Jin and Zhao, 2000), but no information is available regarding the ability of WB.

Thus, it might be that the proposed mechanisms for the use of dietary fibre in the diet, which would promote the growth and modulation of a symbiotic microbiota, would be inconsistent with the commercial use of in-feed antimicrobials or ZnO, which reduces digesta fermentation in the GIT. Moreover, some fibrous ingredients derived from cereal grains, such as wheat bran or dry distillers' grains; contain remarkable amounts of phytic acid, a strong chelator of important minerals such as calcium, magnesium, iron and zinc (O'Dell and Savage, 1960).

In the present study we designed three experiments to evaluate: 1.- the likely role of WB and other fibre sources on their ability to bind *E. coli in-vitro* (Experiment 1). 2.- the effects of including WB and/or ZnO in the diet of newly weaned piglets on the productive performance and the microbial activity in the GIT (Experiment 2); and finally 3.- the likely interactions which may be established *in-*

vitro between the WB and ZnO in the intestinal digesta and with respect to the *E. coli* growth (Experiment 3).

7.2. Materials and methods

7.2.1. Experiment 1: *In-vitro* adhesion test

7.2.1.1. Fibrous ingredients

Seven different fibrous ingredients: wheat bran, rice hulls, soybean hulls, oat hulls, pea hulls, sugar beet pulp and cereal straw were selected as test products. BSA (Sigma, St Louis) served as proteinaceous reference (negative control) following the protocol described by Becker et al. (2007).

7.2.1.2. Bacterial strains

Two different E.*coli* strains were used in this experiment to elucidate the interaction between the fibre substrates and the bacterial fimbriae. The first one was an *E. coli* K88 ETEC (strain FV12048) isolated from a colibacillosis outbreak in Spain (Blanco et al., 1997), serotype (O149:K91:H10, F4+, LT1+, STb+) that was provided by the *E. coli* Reference Laboratory, Veterinary Faculty of Santiago de Compostela (Lugo, Spain). The other strain was a non-fimbriated *E. coli* (F4 -, F6 -, F18 -, LT1 -, ST1 -, ST2 +, Stx2e -) isolated from the faeces of post-weaning piglets and kindly donated by the Department of Animal Health and Anatomy from the Universitat Autònoma de Barcelona.

Bacteria were cultured in unshaken Luria broth (Sigma, St Louis) at 37ºC and serial passage every 48h, at least five times. Bacterial cells from the culture were harvested and processed as earlier described (Becker et al., 2007).

7.2.1.3. *In-vitro* adhesion test

E. coli K88 and the non-fimbriated strain were allowed to adhere to different fibre components supplied as well coatings in microplates in a miniaturized adhesion test, following the protocol described by Becker et al. (2007). Briefly, fibre ingredients were suspended in PBS to a final concentration of 4% (w/v). The suspensions were sonicated three times for 30s each (Unheated Ultrasonic Bath

MU series, Clifton, Nickel Electro Ltd, Weston-super-Mare, UK) and then centrifuged at 460 x g for 5 minutes (Mikro 220R, Hettich Instruments, Germany). For coating, totals of 350 µL supernatant per well were pipetted into the flat-bottom wells of high-binding polystyrene microtitration plates (Microlon F plate 655 092; Greiner Bio-One BV, Alphen a/d Rijn, The Netherlands). Subsequently, plates were incubated overnight at 4ºC. Wells coated with 1% BSA in PBS were included as negative controls in each plate. Plates were washed with 350 µL PBS to remove non-binding material. Afterwards, blocking of non-specific sites of adhesion was done by incubating the plates with 350 µL per well of 1% BSA in PBS (w/v) that contained 0.5% sodium azide at 4ºC for 1h. Thereafter, plates were washed twice with 350 µL PBS. Bacteria were added as 300 µL volumes to the microtitration plate wells after growth, washing and suspending in PBS to a final concentration of 1.20 x 10^8 CFU/mL. Bacteria were allowed to adhere by incubation at room temperature for 30 min. Afterwards, the wells were washed three times with 300 µL PBS to remove non-adherent bacteria. Plates also included wells without the bacteria addition step to control possible contamination of the fibre substrates with naïve bacteria. Bacteria were allowed to growth in Luria broth media by incubation in a microplate reader (SPECTRAmax 384 Plus, Molecular Devices Corporation, Sunnyvale, California, USA) at 37ºC. Bacterial growth was monitored as optical density (OD) at 650 nm at intervals of 10 minutes. All readings were done in two independent assays and in triplicate per assay. The test principle as it is described by Becker et al. (2007) is based on an inverse relationship between initial cell densities and the appearance of growth: the higher the adhering cell numbers, the shorter the detection times of growth.

7.2.2. Experiment 2: *In-vivo* experiment

7.2.2.1. Animals and diets

This experiment was performed at the Animal Facilities of the Universitat Autònoma de Barcelona and received prior approval from the Animal Protocol Review Committee of this institution. The treatment, management, housing, husbandry and slaughtering conditions conformed to the European Union Guidelines (The Council of the European Communities, 1986).

Table 7.1. Composition and chemical analysis of pre-starter diets (g/kg dry matter) (Experiment 2: *In-vivo* experiment).

	Diets[a]			
	CT	WB	ZnO	WB-ZnO
Ingredients				
Corn	414.0	367.0	414.0	364.0
Barley	200.0	200.0	200.0	200.0
Whey	112.0	102.0	112.0	102.0
High fat whey	69.0	90.0	69.0	90.0
Soybean protein concentrate	55.0	52.0	55.0	52.0
Spray dried porcine plasma	50.0	50.0	50.0	50.0
Wheat gluten	30.0	30.0	30.0	30.0
Fish meal LT [b]	40.0	40.0	40.0	40.0
Wheat bran	-	40.0	-	40.0
Calcium carbonate	10.0	10.0	10.0	10.0
Dicalcium phosphate	7.0	7.0	7.0	7.0
Benzoic acid	5.0	5.0	5.0	5.0
Synthetic amino acids [c]	11.2	11.2	11.2	11.2
Vitamin and mineral premix [d]	3.7	3.7	3.7	3.7
Zinc oxide	-	-	3.0	3.0
Chemical analysis				
Dry matter	902.0	903.0	902.0	903.0
Gross energy (MJ/Kg)	17.8	17.9	17.8	17.9
Crude protein (CP; N x 6.25)	203.0	200.0	203.0	200.0
Neutral detergent fibre	79.0	92.0	79.0	92.0
Acid detergent fibre	23.0	28.0	23.0	28.0
Ether Extract	63.0	79.0	63.0	79.0
Ash	56.0	57.0	56.0	57.0

[a]Diets: CT, control diet; WB, wheat bran diet; ZnO, zinc oxide diet and WB-ZnO, wheat bran and zinc oxide diet.
[b]Fish meal low temperature: product obtained by removing most of the water and some or all of the oil from fish by heating at low temperature (<70ºC) and pressing.
[c]Synthetic amino acids: L-Lysine 0.99, DL-Methionine 0.99, L-Tryptophan 0.10, L-Threonine 0.98.
[d]Supplied per kilogram of feed: 5000 IU of vitamin A, 1000 IU of vitamin D3, 15.0 mg of vitamin E, 1.3 mg of vitamin B1, 3.5 mg of vitamin B2, 1.5 mg of vitamin B6, 0.025 mg of vitamin B12, 10.0 mg of calcium pantothenate, 1.3 g of coline chloride, 15.0 mg of niacin, 15.0 mg of biotin, 0.1 mg of folic acid, 2.0 mg of vitamin K3, 80.0 mg of Fe, 6.0 mg of Cu, 0.7 mg of Co, 60.0 mg of Zn, 30.0 mg of Mn, 0.7 mg of I, 0.1 mg of Se and 0.15 mg of etoxiquin.

A total of 64 commercial crossbred piglets ((Large White x Landrace) x Pietrain), which had been excluded from receiving creep feed, were weaned at 21 days old with an average body weight (BW) of 6.7 ± 0.37 kg. Pigs were transported from a commercial farm to the animal facilities and placed into thirty-two pens (two animals per pen). Each pen had a feeder and a water nipple to ensure *ad libitum* feeding and

free water access. The pens were allotted to four dietary treatments (eight replicates for each treatment, Table 7.1) in a 2×2 factorial arrangement that included two levels of WB (0 *v*. 40 g/kg, CT *v*. WB, respectively) and two levels of ZnO (0 *v*. 3 g/kg, 0 *v*. ZnO diet, respectively) in the diet. The diets were prepared based on ground corn, barley, and soybean protein concentrate.

7.2.2.2. Experimental procedures and sampling

Animals received the diets from day 1 to day 12 of the experiment. Individual BW and pen feed consumption were recorded on days 0, 3, 6, 9 and 12 after weaning. Physical and behavioural examination of the animals was done daily to evaluate their health status. Samples of fresh faeces were collected from the rectum of one animal per pen for microbial counts on day 3, 6, 9 and 12 after weaning. At the end of the experimental period, faecal samples were kept in tubes and immediately frozen at -80ºC for lactobacilli quantification and short chain fatty acids (SCFA) analyses.

7.2.2.3. Analytical procedures

Chemical analyses of the diets (Table 7.1) were performed according to the Association of Official Analytical Chemists (AOAC, 1995) standard procedures.

Traditional culture methods were used to determine some bacterial groups. Immediately after collection of the faeces samples, each one was diluted 1/10 in PBS (Sigma, St Louis) and subsequently homogenized. Viable counts of enteroccoci were done by plating serial 10-fold dilutions onto Chromocult® Enterococci-Agar (Merck K GaA, Darmstadt, Germany) and incubating the plates for 24 h at 37ºC. For the enumeration of *E. coli* and coliform, 1 mL of solution of the corresponding dilution was pipetted onto an *E. coli-coliform* count plate (3M Petrifilm, Europe Laboratories 3M Santé, Cergy-Pontoise, France) with Violet Red Bile gel as an indicator of glucuronidase activity. The plates were incubated for 48 h at 35ºC, and all blue *E. coli* and red and blue coliform colonies were counted following the manufacturer's instructions. DNA from faeces was extracted and purified using the commercial QIAamp DNA Stool Mini Kit (Qiagen, West Sussex, UK) and the lactobacilli population was quantified by real time PCR using SyBR

Green dye, following the protocol described by Castillo et al. (2006). The t-RFLP analysis of bacterial community was performed following the procedure described by Hojberg et al. (2005) and adapted by Castillo et al. (2006). The analysis of t-RFLP data was carried out following Castillo et al. (2006). Briefly, sample data consisted of size and peak area for each TRF. Richness was considered as the number of peaks in each sample after standardization. For pair-wise comparison of the profiles, a Dice coefficient was calculated and dendrograms were constructed using Fingerprinting II (Informatix, Bio-Rad, CA, USA) software and an un-weighted pair-group method with averaging algorithm (UPGMA).

Finally, SCFA concentrations were determined by gas chromatography, after submitting the samples to an acid-base treatment followed by ether extraction and derivatization, as described by Jensen and Jorgensen (1994).

7.2.3. Experiment 3: *In-vitro* wheat bran and zinc oxide interaction test

7.2.3.1. Sample preparation

In order to elucidate the interaction between WB and ZnO and the likely role of phytates, eight different samples were prepared in a 4 x 2 factorial design, which included four different buffered solutions (a negative control; 4% WB; 4% WB + 0.02% phytase enzyme (Ronozyme® P500, DSM Nutritional Products Ltd., US, 5000 IU/g); and 4% WB + 0.02% xylanase and glucanase enzyme mixture (Rovabio™ Excel AP, Adisseo, France, 22000 IU xylanase and 2000 IU glucanase/g)), and two levels of ZnO (0 v. 0.3% w/v). Samples of buffered solutions were adjusted to a pH of 5.1 with HCl and incubated for 4 hours at room temperature. After that, the suspensions were sonicated three times for 30s each and then centrifuged at 460 x g for 5 minutes. The supernatant obtained was adjusted to pH of 7.0 with NaOH and ZnO added appropriate to the specific treatment.

7.2.3.2. Bacterial strains

Two different *E. coli* strains (*E. coli* K88 and a non-fimbriated *E. coli* strain) were used in this experiment as described above.

7.2.3.3. *In-vitro* test

E. coli K88 and the non-fimbriated *E. coli* strains were centrifuged (1700 x g) and adjusted to a final concentration of approximately 3.5 - 3.9 x 10^8 CFU/mL in LB. Subsequently, 750 µL of each bacterial suspension were incubated with 750 µL of each experimental treatment. Thereafter, 300 µL of each suspension were added to polystyrene microtitration plates and the growth of the bacteria measured in a microplate reader at 37ºC following the protocol described by Becker et al. (2007). Bacterial growth was monitored as optical density (OD) at 650 nm at intervals of 10 minutes for 10 hours. All readings were done in two independent assays and in triplicate per assay.

7.2.4. Statistical analyses

All data from the *in-vivo* experiment was subjected to ANOVA using the GLM procedure of SAS (SAS Inst., Inc., Cary, NC, USA). Data was analyzed as a 2 x 2 factorial arrangement of treatments, with WB and ZnO as the factors.

The OD data from the *in-vitro* experiments were processed by nonlinear regression analysis and the P-NLIN (Gauss-Newton method) procedure of SAS (SAS Inst., Inc., Cary, NC, USA) following the equations described by Becker et al. (2007). The least square means for the $t_{OD=0.05}$ (h) results for the adhesion *in-vitro* tests (Experiment 1 and 3) were analyzed as a factorial arrangement of treatments, with fibre source or treatment and *E. coli* strain as the factors.

Results are presented as least square means. Differences were considered significant at $P < 0.05$. Tendencies for $0.05 < P < 0.15$ were also presented.

7.3. Results

7.3.1. Experiment 1: *In-vitro* adhesion test

Table 7.2 presents the detection times of growth for *E. coli* K88 and for the non-fimbriated *E. coli* as the duration (h) needed for the cultures to reach an OD of 0.05 at 650 nm. In the present study, an interaction was found between the *E. coli* strain and the fibre source ($P = 0.0001$). Significant differences between the fibre substrates were found related to the adhesion of the two *E. coli* strains. The *E. coli* K88 adhered more strongly ($P = 0.0001$) to the WB compared to the other fibre

substrates and the negative control treatment. Similarly, non-fimbriated *E. coli* showed a higher attachment (P = 0.0001) to the WB substrate compared to soybean hulls, sugar beet pulp, oat hulls and the negative control treatment.

Table 7.2. Detection times of bacterial growth $t_{OD=0.05}$ (h) for *E. coli* K88, non-fimbriated *E. coli*, as a measure for adhesion in different fibre ingredients (Experiment 1: *In-vitro adhesion test*).

Product	$t_{OD=0.05}$ *E. coli* K88	$t_{OD=0.05}$ non-fimbriated *E. coli*
Wheat bran 4%	0.94 ^{x}	2.73 ^{x}
Rice hulls 4%	2.74 ^{y}	2.88 ^{xy}
Soybean hulls 4%	3.11 ^{y}	3.27 ^{yz}
Cereal straw 4%	3.12 ^{y}	3.01 ^{xyz}
Sugar beet pulp 4%	3.22 ^{y}	3.36 ^{yz}
Pea hulls 4%	3.00 ^{y}	3.11 ^{xyz}
Oat hulls 4%	2.69 ^{y}	3.43 ^{z}
Negative control	2.92 ^{y}	3.34 ^{yz}
SEM	0.193	
P – values		
Fibre product	0.0001	
Bacteria	0.0001	
Fibre product x Bacteria	0.0001	

The data represent least-squared means. Detection time means marked by different letters (x,y,z) are significantly different within the same column (P < 0.05). Products with the shortest detection time bound most cells of the bacterial type.

7.3.2. Experiment 2: *In-vivo* experiment

7.3.2.1. Animal performance and health status

The effects of WB and ZnO on the average daily feed intake (ADFI) and average daily gain (ADG) of the animals as well as the incidence of diarrhoea are shown in Table 7.3. The inclusion of ZnO in the diet increased the ADFI of the animals from day 6 to 12 (P = 0.006) and from day 0 to 12 (P = 0.035). This resulted in an increased ADG of the animals for the same periods (P = 0.008 and P = 0.036, respectively) and a higher BW at the end of the experiment (P = 0.044) compared to the animals not receiving ZnO in the feed. Inclusion of ZnO in the diet also reduced the incidence of diarrhoea (P = 0.009).

Table 7.3. Body weight (BW), average daily feed intake (ADFI), average daily gain (ADG) and diarrhoea incidence in early weaned pigs (Experiment 2: *In-vivo* experiment).

Item	Diets [a]				SEM	P-values [b]		
	CT	WB	ZnO	WB-ZnO	(n= 8)	WB	ZnO	WBxZnO
Body Weight (g)								
Day 0	6700	6725	6742	6698	53.7	0.735	0.783	0.217
Day 6	6859	7014	7130	6948	249.6	0.918	0.428	0.202
Day 12	7933	8120	8583	8350	390.2	0.908	0.044	0.303
ADFI (g/animal and day)								
0 - 6 d	74.9	91.0	105.1	75.3	25.41	0.603	0.578	0.096
6 - 12 d	278.8	279.9	358.9	321.6	39.91	0.333	0.006	0.331
0 - 12 d	176.8	184.9	231.9	198.5	28.89	0.397	0.035	0.175
ADG (g/animal and day)								
0 - 6 d	6.6	12.1	16.2	10.4	10.15	0.977	0.452	0.294
6 - 12 d	44.8	46.1	60.6	58.4	8.83	0.925	0.008	0.703
0 - 12 d	25.7	29.1	38.4	34.4	7.62	0.936	0.036	0.359
Diarrhea (nº animals)								
0 - 12 d	14/16	16/16	7/16	11/16	0.41	0.735	0.009	0.319

Different superscripts (x, y) in the same row denote significant differences ($P < 0.05$)
[a]Diets: CT, control diet; WB, wheat bran diet; ZnO, zinc oxide diet and WB-ZnO, wheat bran and zinc oxide diet.
[b]P-values: WB, effect of WB inclusion in the diet; ZnO, effect of inclusion or not ZnO in the diet; WBxZnO, effect of WB and ZnO interaction in the diet.

7.3.2.2. Metabolic activity and composition of faecal microbiota

Concentrations of total and individual SCFA in faecal samples and also counts of major bacterial groups, using traditional microbiology or qPCR are shown in Table 7.4. Significant differences were observed for the SCFA concentration associated with the incorporation of WB and ZnO into the diet. Moreover, interaction between WB and ZnO was also significant for the total SCFA ($P = 0.048$), the propionic acid ($P = 0.018$) and the butyric acid concentration ($P = 0.007$), and also tended to be significant ($P = 0.120$) for acetic acid. Thus, the WB diet increased the total SCFA concentration ($P = 0.011$), propionic acid ($P = 0.014$) and butyric acid ($P = 0.027$) in comparison to the CT, ZnO and WB-ZnO diets. The incorporation of WB (WB and WB-ZnO diets) increased the concentration of isoacids ($P = 0.001$) and the inclusion of ZnO (ZnO and WB-ZnO diets) diminished the concentrations of acetic ($P = 0.024$) and isoacids ($P = 0.001$).

Table 7.4. Total and profile of short-chain fatty acids (micromol/ g FM) in day 12 after weaning, enteroccoci and *E. coli* counts (log CFU/ g FM) and lactobacilli population (log copies gen 16S rDNA/ g FM) in the faeces of piglets early after weaning (Experiment 2: *In-vivo* experiment).

	Diets [a]				SEM	P-values [b]		
	CT	WB	ZnO	WB-ZnO	(n = 8)	WB	ZnO	WBxZnO
Short-chain fatty acids								
Total	71.6 ^{y}	109.7 ^{x}	59.5 ^{y}	65.1 ^{y}	18.41	0.011	0.002	0.048
Acetic	42.2	56.9	37.8	35.7	12.93	0.241	0.024	0.120
Propionic	17.8 ^{y}	29.2 ^{x}	14.6 ^{y}	14.8 ^{y}	5.32	0.014	0.001	0.018
Butyric	9.5 ^{y}	18.3 ^{x}	10.2 ^{y}	9.2 ^{y}	3.98	0.027	0.019	0.007
Isoacids	4.3	5.1	1.9	3.2	0.91	0.001	0.001	0.494
Microbial population								
Day 3								
Enteroccoci	6.1	5.9	5.4	5.8	0.71	0.721	0.299	0.405
E. coli	6.6	6.2	6.1	6.5	0.89	0.988	0.923	0.418
Coliforms	6.9	6.6	6.2	6.8	0.95	0.720	0.573	0.434
Day 6								
Enteroccoci	5.8	5.8	6.9	5.9	0.73	0.166	0.158	0.186
E. coli	4.9 ^{xy}	4.3 ^{xy}	2.6 ^{y}	5.0 ^{x}	1.11	0.154	0.201	0.026
Coliforms	4.9	4.4	3.6	5.1	0.97	0.356	0.523	0.068
Day 9								
Enteroccoci	6.8	6.3	6.8	5.8	0.56	0.016	0.405	0.418
E. coli	5.0 ^{xy}	3.9 ^{y}	4.2 ^{xy}	5.3 ^{x}	0.87	0.986	0.515	0.024
Coliforms	5.1 ^{xy}	4.1 ^{y}	4.7 ^{xy}	5.4 ^{x}	0.72	0.580	0.229	0.033
Day 12								
Enteroccoci	6.3	5.9	6.8	6.7	0.65	0.357	0.064	0.589
E. coli	6.6 ^{x}	5.2 ^{y}	5.6 ^{xy}	5.9 ^{xy}	0.72	0.148	0.700	0.034
Coliforms	6.7 ^{x}	5.4 ^{y}	5.7 ^{xy}	6.1 ^{xy}	0.63	0.222	0.862	0.028
Lactobacilli	11.9	11.7	11.1	11.5	0.47	0.761	0.084	0.532

Different superscripts (x, y) in the same row denote significant differences ($P < 0.05$)
[a]Diets: CT, control diet; WB, wheat bran diet; ZnO, zinc oxide diet and WB-ZnO, wheat bran and zinc oxide diet.
[b]P-values: WB, effect of WB inclusion in the diet; ZnO, effect of inclusion or not ZnO in the diet; WBxZnO, effect of WB and ZnO interaction in the diet.

In order to identify differences promoted by the diets between the major bacterial groups of relevance in disbiosis during the post-weaning period, microbial counts were included in the study using culturing methods or qPCR (Table 7.4). A significant interaction was observed between the ZnO and the WB supplementation on the counts of *E. coli* and coliforms after weaning. The simultaneous incorporation of ZnO and WB in the diet increased the *E. coli* and coliforms counts as compared to the ZnO diet on day 6 after weaning ($P = 0.026$) and as compared to the WB diet

on day 9 after weaning (P = 0.024), showing a negative interaction between ZnO and WB in the post-weaning diets. On day 12, animals fed the WB diet showed lower counts of *E. coli* (P = 0.034) and coliforms (P = 0.028) than the CT diet but no significant differences were observed with the ZnO or ZnO-WB diet. On day 12, the incorporation of ZnO in the diets (ZnO and WB-ZnO) also tended to increase the enteroccoci population (P = 0.064) and to reduce the lactobacilli counts (P = 0.084) in the faeces of 12 days weaned pigs as compared to animals that did not receive ZnO in the diet (CT and WB).

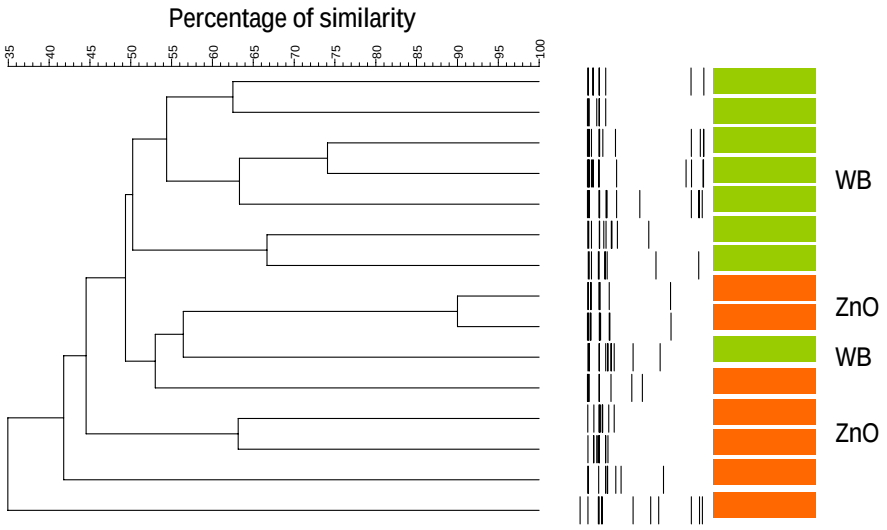


Fig. 7.1. Dendogram illustrating the correlation between experimental diets: 4 % wheat bran diet (WB) and 0.3 % zinc oxide diet (ZnO), in t-RFLP banding patterns of faeces of post weaning piglets. The dendogram distances are in percentage of similarity (Experiment 2: *In-vivo* experiment).

Finally, to evaluate global changes in the microbial ecosystem, the t-RFLP method was employed. Fig. 7.1 shows the analysis focused on two of the diets: the WB and the ZnO diets. It shows the microbial profiles of all pens except one from which we were unable to take faeces samples. The effect of the diet on the composition of faeces was clearly observed as most of the animals were grouped in two separate clusters. Microbial profiles of WB pigs were more similar (50-75%)

than those of ZnO pigs which showed more heterogeneous microbial profiles (52 – 90%).

7.3.3. Experiment 3: The *in-vitro* analysis of interaction between wheat bran and zinc oxide

Table 7.5 presents the results related to Experiment 3 as detection times of growth for *E. coli* K88 and non-fimbriated *E. coli* as the duration (h) needed for the cultures to reach an OD of 0.05 at 650 nm. A significant (P = 0.0001) interaction between the ZnO and the buffered solutions was found related to the growth of the two *E. coli* strains. The incorporation of ZnO in the buffered solution inhibited (P = 0.0001) the bacterial growth for both *E. coli* strains in comparison to the negative control. Also the ZnO supplementation showed antimicrobial effects when supplemented into the WB + phytase treatment. However, when it was added to the WB or WB + xylanase treatment ZnO did not reduce the growth of *E. coli*.

Table 7.5. Detection times of bacterial growth $t_{OD=0.05}$ (h) for *E. coli* K88, non-fimbriated *E. coli*, as a measure of the ability of the *E. coli* strains to grow on different substrates (Experiment 3: *In-vitro* wheat bran and zinc oxide test).

Items[a]	ZnO inclusion[b]	$t_{OD=0.05}$ *E. coli* K88	$t_{OD=0.05}$ non-fimbriated *E. coli*
Negative control	-	0.22 ^{x}	0.24 ^{x}
	+	--- ^{v}	--- ^{y}
WB	-	0.50 ^{y}	0.20 ^{x}
	+	0.42 ^{y}	0.35 ^{x}
WB + phytase	-	1.10 ^{z}	0.45 ^{x}
	+	1.53 ^{v}	--- ^{y}
WB + xylanase	-	0.33 ^{xy}	0.17 ^{x}
	+	0.50 ^{y}	0.48 ^{x}
SEM		0.502	
P – values			
Buffered solutions		0.0046	
Zinc oxide		0.0001	
Buffered solutions x Zinc oxide		0.0001	

[a]Negative control was based on PBS; the WB inclusion was at a level of 4%, and the phytase and xylanase inclusion at a level of 0.02% (w/v).
[b]ZnO inclusion was at a level of 0.3% (w/v).
The data represent least-squared means. (---) Total inhibition of the bacterial growth. Detection time means marked by different letters are significantly different within the same column (P < 0.05).

7.4. Discussion

7.4.1. Potential of different fibrous substrates to bind *E. coli*

Different studies have shown the promising effects of glycoconjugates from different origins such as cranberry and blueberry extracts (Ofek et al., 1996), mannan-oligosaccharides (MOS) (Spring et al., 2000; Fernandez et al., 2002), palm kernel extracts (Allen et al., 1997) or soya and fermented soya bean products (Kiers et al., 2002) to inhibit the adhesion of different pathogens such as *E. coli* or Salmonella to the intestinal mucosa of different animal species. Dietary fibre from plants may provide an alternative adhesion matrix to enteropathogenic bacteria because of their carbohydrate nature similar to the intestinal receptors of such pathogens and low digestibility. Becker and Galleti (2008) tested the binding capacity of different food and feed components for *E. coli* K88, *S. enterica* sv. Thypimurium and *Lactobacillus spp*. isolated from pigs, chickens, calves and human subjects. They reported positive scores for sesame seed extract and soya bean product against *E. coli* K88 *in-vitro*. In recent studies, Kim et al. (2008) and Becker et al. (2009) also reported the blocking capacity of oat hulls or pea hulls against *E. coli* K88. In our study, WB extracts showed the highest ability to bind *E. coli* K88 among the different fibre sources evaluated. The binding activity was higher in the presence of the F4 fimbriated *E. coli* K88 in comparison to the non-fimbriated *E.* coli. These results are in good agreement with those we found earlier regarding the reduction promoted by the WB on enterobacteria and coliforms counts in digesta and attached *E. coli* K88 to the ileum mucosa (Molist et al., 2009a, b). WB is one of the more available fibre sources for human and animal feeding. It contains insoluble non-starch polysaccharides (Ralet et al., 1990) mainly as arabinoxylan, cellulose and β-glucan, but also minute levels of glucomannans (Mares and Stone, 1973) and arabinogalactans (Fincher et al., 1974) originating from the aleurone and endosperm cells. It might be speculated that the soluble fraction of WB, may form a matrix in the gut in which fimbriated *E. coli* is captured. The adhesion of bacteria to the WB matrix may allow their growth, as is observed in the *in-vitro* system, but it also provides a mechanism by which the attachment and proliferation of *E. coli* K88 at the intestinal epithelium is inhibited or reduced.

7.4.2. The influence of wheat bran and zinc oxide on the adaptation of piglets after weaning

Dietary fibre has become one of the dietary components attracting most interest for the nutrition of young animals. The hypothesis is that young animals may have a minimum requirement of fibre for an adequate functionality of the digestive tract, and a proper development of the intestinal microbiota. However, few studies have explored the influence of fibre ingredients in the diets of early weaning animals when in-feed antimicrobials are also incorporated in practical conditions. In our study, the incorporation of WB did not improve the animal performance, as was also shown in earlier studies (Molist et al., 2009a, 2010a). On the other hand, dietary supplementation with a high level of ZnO (3 g/kg) increased the feed intake and the ADG of the animals and reduced the onset of diarrhoea in weanling piglets during the first days after weaning. These results are in good accordance with observations from animal performance studies, in which a larger number of animals were used (Hill et al., 2000; Case and Carlson, 2002).

Since ZnO is known to possess antimicrobial properties, it has been usually assumed that it enhances growth by controlling pathogenic bacteria or by mechanisms resembling those of antibiotics. In our study, ZnO reduced the fermentation activity and the counts of lactobacilli and increased the counts of enterococci. Our results are in agreement with those presented by Hojberg et al. (2005). These authors suggested that a reduction in the gram positive commensal bacteria in the proximal part of the GIT may provide more energy for the host animal and contribute to the growth-promoting effect of high dietary ZnO doses. In contrast, other reports suggest other mechanisms of action based on the fact that piglets may have an extraordinary high requirement of zinc early after weaning (Carlson et al., 2004; Li et al., 2006). Some studies have demonstrated that high doses of ZnO are effective in increasing the feed intake and in modulating the gene expression of the animals (Martinez et al., 2002; Ou et al., 2007). Yin et al. (2009) observed that ZnO supplementation increased plasma levels of ghrelin in early weaned piglets. Ghrelin is a hormone released by the stomach, which is involved in the secretion of growth hormone and IGF-I, and in the stimulation of the feed intake and muscle growth.

Our results also revealed a decrease in the incidence of diarrhoea and the counts of *E. coli* in faeces with the ZnO supplementation, as observed by Cardinal et al. (2006). Diarrhoea in piglets is an important problem which is associated, in some cases, with an over-proliferation of enteropathogenic *E. coli*. However, diarrhoea may also reflect the reduced absorptive capacity of the GIT of the young piglets. In the present study, most of the animals presented diarrhoea without a pathological picture of fever, dehydration or apathy. Inadequate feed intake during the immediate post-weaning period induces intestinal inflammation and compromises the villus-crypt structure and function (McCracken et al., 1999). Mast cells play an important role in this process (Abbas et al., 1991). Ou et al. (2007) demonstrated that zinc was able to reduce the number of mast cells in the small-intestinal mucosa and submucosa and inhibited histamine release from mast cells.

In contrast to the results observed with the ZnO treatments, the inclusion of a moderate level of WB in the diet increased the concentration of fermentation products in the faeces, especially of butyrate. Butyrate is considered the principal oxidative fuel for the colonocytes and may have beneficial tropic effects on the inflamed caeco-colonic mucosa (Oufir et al., 2000). It is accepted that starch and bran from wheat or oats stimulate the formation of butyrate (Hojberg et al., 2005). As observed in previous studies with insoluble fibre sources (Molist et al., 2009a, 2009b, 2010a; Kim et al., 2008), the addition of WB in the diet decreased the *E. coli* and coliform bacteria counts in the faeces. However, a significant negative interaction between the WB and the ZnO supplementation was observed. The supplementation with ZnO decreased the concentration of SCFA when WB was included in the diet, and likely diminished through this way the effect of WB on the coliform bacteria counts in the faeces. However, it is also intriguing that the combination of WB and ZnO also reduced the effect of therapeutic doses of ZnO in the counts of *E. coli* and coliform in faeces. Experiment 3 was designed to confirm the observed interactions between WB and ZnO on the coliforms growth *in-vitro*, and to define the possible mechanisms involved.

7.4.3. Possible mechanism involved in the interaction between wheat bran and zinc oxide

Negative interactions between WB and ZnO have been reported in this study *in-vivo* and *in-vitro*. In the i*n-vivo* experiment the combination WB-ZnO did not reduce the *E. coli* and the coliforms counts on day 6 and 9 post-weaning compared to the ZnO or WB diet. In the *in-vitro* experiment the combination WB-ZnO had neither the same antimicrobial effect on the *E. coli* strains as ZnO nor did the combination WB-phytase and ZnO. Therefore, it is suggested that a negative interaction between phytic acid and ZnO modifies the antimicrobial properties of therapeutic doses of ZnO *in-vivo* and *in-vitro*. Champagne and Fisher (1990) suggested that phytic acid (PA), primarily found in the pericarp of the cereal grains, may form a rather stable complex with bivalent cations, such as the Cu^{2+} and Zn^{2+}, that precipitates at Zn:phytate molar ratios of 3.5 to 4:1. These complexes are known not only to affect the Zn bioavailability, but also to decrease the availability of the phytate for the hydrolysis by phytase (Augspurger et al., 2004). O´Dell and Savage (1960) first reported a decreased availability of Zn in chicks fed phytate. In human diets, Hambidge et al. (2008) have recently reported that the amount of dietary Zn required to attain 6.4 mg absorbed Zn/d goes from 40 mg dietary Zn at zero phytate intake to 100 mg dietary Zn with a dietary phytate intake of 900 mg/d.

Procedures that degrade phytate have been studied as a means to increase the bioavailability of zinc and other cations in the diet and therefore increase the nutritional value of the meal (Bobilya et al., 1991). It is known that fermentation of feed may reduce the PA:Zn ratio, promoting a better Zn absorption (Hirabayashi et al., 1998). Other authors, such as Gaetke et al. (2009) also have shown that yogurt (both active and heat-treated) protects against growth retardation in weanling rats fed high PA. In the animal, feeding PA has been regarded as an anti-nutrient which reduces P availability, and most research in this field has been aimed at eliminating PA from the animal feed by adding exogenous phytase to it. The term phytase is defined as a class of phosphatases with the *in-vitro* capability to release at least one phosphate from PA (McCollum and Hart, 1908). Some authors (Martinez et al., 2004; Revy et al., 2005) have suggested that phytase supplementation may increase the amount of Zn absorbed, even when pharmacological doses of Zn are

included in the diet. Thus, Martinez et al. (2004) suggested that current pharmacological doses of Zn (2000 mg/kg) fed to pigs could be reduced to 1000 mg/kg by adding phytase. This was shown by an overall greater metallothionein mRNA abundance and Zn absorption. As far as we know, the present study confirms a negative interaction between the WB and therapeutic doses of ZnO in their effects on the population of coliform bacteria. Taking into account these results, phytase supplementation may be proposed as a good approach to increase the effectiveness or reduce the levels of ZnO in the post-weaning diets.

7.5. Conclusion

Based on the results of the present work, we conclude that the incorporation of WB in the diet of early weaning piglets may improve their gut health by modulating the activity of the intestinal microbiota, enhancing the fermentation and blocking the attachment of *E. coli* K88 to the intestinal mucosa. A negative interaction observed *in-vivo* and *in-vitro* between WB (rich in phytate) and ZnO, make it necessary to consider the inclusion of phytase enzymes in early weaning diets.

7.6. Acknowledgments

This research was supported by the Spanish CICYT (project AGL2005-07438-C02-01). We thank the Ministerio de Educación, Cultura y Deporte, Spain for research fellowships. We also thank the Servei de Granges i Camps Experimentals de la UAB for their service and assistance during the experiment.

7.7. References

Abbas, A.K., Lichtman, A.H., Pober, J.S., 1991. Cytokines, in: Abbas, A.K., Lichtman, A.H., Pober, J.S., (Eds.), Cellular and Molecular Immunology. W.B. Saunders Company, Philadelphia.

Allen, V.M., Fernandez, F., Hinton, M.H., 1997. Evaluation of the influence of supplementing the diet with mannose or palm kernel meal on Salmonella colonisation in poultry. Br. Poul. Sci. 38, 485-488.

AOAC, 1995. In: Association of Official Analytical Chemists (Ed.), Official Methods of Analysis. Arlington, VA, USA.

Augspurger, N.R., Spencer, J.D., Webel, D.M., Baker, D.H., 2004. Pharmacological zinc levels reduce the phosphorus-releasing efficacy of phytase in young pigs and chickens. J. Anim. Sci. 82, 1732-1739.

Ball, R.O., Aherne, F.X., 1987. Influence of dietary nutrient density, level of feed intake and weaning age on young pigs. II. Apparent nutrient digestibility and incidence and severity of diarrhea. Can. J. Anim. Sci. 67, 1105-1115.

Becker, P.M., Galleti, S., Roubos-van den Hil, P.J., van Wikselaar, P.G., 2007. Validation of growth as measurand for bacterial adhesion to food and feed ingredients. J. Appl. Microbiol. 103, 2686-2696.

Becker, P.M., and Galleti, S., 2008. Food and feed components for gut health-promoting adhesion of *E. coli* and *Salmonella enterica*. J. Sci. Food Agric. 88, 2026-2035.

Becker, P.M., van Wikselaar, P.G., Jansman, A.J.M., van der Meulen, J., 2009. Pea dietary fiber for adhesion and excretion of enterotoxigenic *E. coli* K88 to prevent intestinal colonization. J. Anim. Sci. 87, 172.

Bertschinger, H.U., Moon, H.W., Whipp, S.C., 1972. Association of Escherichia coli with the small intestinal epithelium. I. Comparison of enteropathogenic and nonenteropathogenic porcine strains in pigs. Infect. Immun. 5, 595-605.

Bikker, P., Dirkzwager, A., Fledderus, J., Trevisi, P., Le Huërou-Luron, I., Lallès, J.P., Awati, A., 2006. The effect of dietary protein and fermentable carbohydrates levels on growth performance and intestinal characteristics in newly weaned piglets. J. Anim. Sci. 84, 3337-3345.

Blanco, M., Blanco, J.E., Gonzalez, E.A., Mora, A., Jansen, W., Gomes, T.A., Zerbini, L.F., Yano, T., de Castro, A.F., Blanco, J., 1997.Genes coding for enterotoxins and verotoxins in porcine Escherichia coli strains belonging to different O:K:H serotypes: relationship with toxic phenotypes. J. Clin. Microbiol. 35, 2958-2963.

Bobilya, D.J., Ellersieck, M.R., Gordon, D.T., Veum, T.L., 1991. Bioavailabilities of zinc from nonfat dry milk, lowfat plain yogurt, and soy flour in diets fed to neonatal pigs. J. Agric. Food Chem. 39, 1246-1251.

Cardinal, F., D'Allaire, S., Fairbrother, J.M., 2006. Feed composition in herds with or without postweaning *Escherichia coli* diarrhea in early-weaned piglets. J. Swine Health Prod. 14, 10-17.

Carlson, D., Poulsen, H.D., Vestergaard, M., 2004. Additional dietary zinc for weaning piglets is associated with elevated concentrations of serum IGF-I. J. Anim. Physiol. Anim. Nutr. 88, 332-339.

Case, C.L., Carlson, M.S., 2002. Effect of feeding organic and inorganic sources of additional zinc on growth performance and zinc balance in nursery pigs. J. Anim. Sci. 80, 1917-1924.

Castillo, M., Martín-Orúe, S.M., Roca, M., Manzanilla, E.G., Badiola, I., Pérez, J.F, Gasa, J., 2006. The response of gastrointestinal microbiota to avilamycin, butyrate, and plant extracts in early-weaned pigs. J. Anim. Sci. 84, 2725-2734.

Champagne, E.T., Fisher, M.S., 1990. Binding differences of Zn(II) and Cu(II) ions with phytate. J. Inorg. Biochem. 38, 217-223.

Fernandez, F., Hinton, M., Van Gils, B., 2002. Dietary mannan-oligosaccharides and their effect on chicken caecal microflora in relation to Salmonella enteritidis colonization. Avian Pathol. 31, 49-58.

Fincher, G.B., Sawyer, W.H., Stone, B.A., 1974. Chemical and physical properties of an arabinogalactan peptide from wheat endosperm. Biochem. J. 139, 535-545.

Francis, D.H., Grange, P.A., Zeman, D.H., Baker, D.R., Sun, R., Erickson, A.K., 1998. Expression of mucin-type glycoprotein K88 receptors strongly correlates with piglet susceptibility to K88(+) enterotoxigenic Escherichia coli, but adhesion of this bacterium to brush borders does not. Infect. Immun. 66, 4050-4055.

Gaetke, L.M., McClain, C.J., Toleman, C.J., Stuart, M.A., 2009. Yogurt protects against growth retardation in weanling rats fed diets high in phytic acid. J. Nutr. Biochem. 21, 147-152.

Hambidge, K.M., Miller, L.V., Westcott, J.E., Krebs, N.F., 2008. Dietary reference intakes for zinc may require adjustment for phytate intake based upon model predictions. J. Nutr. 138, 2363-2366.

Hampson, D.J., 1994. Escherichia coli in domestic animals, in: School of Veterinary Studies (Eds.), Diarrhoea in Pigs, Murdoch, Australia.

Hedemann, M.S., Eskildsen, M., Laerke, H.N., Pedersen, C., Lindberg, J.E., Laurinen, P., Bach Knudsen, K.E., 2006. Intestinal morphology and enzymatic activity in newly weaned pigs fed contrasting fiber concentrations and fiber properties. J. Anim. Sci. 84, 1375-1386.

Heo, J.M., Kim, J.C., Hansen, C.F., Mullan, B.P., Hampson, D.J., Pluske, J.R., 2009. Feeding a diet with decreased protein content reduces indices of protein fermentation and the incidence of postweaning diarrhea in weaned pigs challenged with an enterotoxigenic strain of Escherichia coli. J. Anim. Sci. 87, 2833-2843.

Hermes, R.G., Molist, F., Ywazaki, M., Nofrarias, M., Gómez de Segura, A., Gasa, J., Pérez, J.F., 2009. Effect of dietary level of proteína and fiber on the productive performance and health status of piglets. J. Anim. Sci. 87, 3569-3577.

Hill, G.M., Cromwell, G.L., Crenshaw, T.D., Dove, C.R., Ewan, R.C., Knabe, D.A., Lewis, A.J., Libal, G.W., Mahan, D.C., Shurson, G.C., Southern, L.L., Veum, T.L., 2000. Growth promotion effects and plasma changes from feeding high dietary concentrations of zinc and copper to weanling pigs (regional study). J. Anim. Sci. 78, 1010-1016.

Hirabayashi, M., Matsui, T., Yano, H., 1998. Fermentation of soybean meal with *Aspergillus usamii* improves zinc availability in rats. Biol. Trace Elem. Res. 61, 227-234.

Hojberg, O., Canibe, N., Poulsen, H.D., Hedemann, M.S., Jensen, B.B., 2005. Influence of dietary zinc oxide and copper sulfate on the gastrointestinal ecosystem in newly weaned piglets. Appl. Environ. Microbiol. 71, 2267-2277.

Houdijk JGM, Hartemink R, Verstegen MWA, Bosch, M.W., 2002. Effects of dietary non-digestible oligosaccharides on microbial characteristics of ileal chyme and faeces in weaner pigs. Arch. Anim. Nutr. 56, 297-307.

Jensen, B.B., Jorgensen, H., 1994. Effect of dietary fiber on microbial activity and microbial gas production in various regions of the gastrointestinal tract of pigs. Appl. Environ. Microbiol. 60, 1897-1904.

Jin, L.Z., Zhao, X., 2000. Intestinal receptors for adhesive fimbriae of enterotoxigenic Escherichia coli (ETEC) K88 in swine-a review. Appl. Microbiol. Biotechnol. 54, 311-318.

Kiers, J.L., Nout, M.J.R., Rombouts, F.M., Nabuurs, M.J.A., van der Meulen, J., 2002. Inhibition of adhesion of enterotoxigenic *Escherichia coli* K88 by soya bean tempe. Lett. Appl. Microbiol. 35, 311-315.

Kim, J.C., Mullan, B.P., Hampson, H.D.J., Pluske, J.R., 2008. Addition of oat hulls to an extruded rice-based diet for weaner pigs ameliorates the incidence of diarrhoea and reduces indices of protein fermentation in the gastrointestinal tract. Br. J. Nutr. 99, 1217-1225.

Li, X., Yin, J., Li, D., Chen, X., Zang, J., Zhou, X., 2006. Dietary supplementation with zinc oxide increases IGF-I and IGF-I receptor gene expression in the small intestine of weanling piglets. J. Nutr. 136, 1786-1791.

Mares, D.J., Stone, B.A., 1973. Studies on wheat endosperm: Chemical composition and ultrastructure of cells walls. Aust. J. Biol. Sci. 26, 793-812.

Martinez, M.M., Hill, G.M., Link, J.E., Greene, J.G., Driksna, D.D., 2002. Effects of pharmacological concentrations of zinc oxide and phytase on zinc excretion and performance in the nursery pig. J. Anim. Sci. 80, 73.

Martinez MM, Hill GM, Link JE, Raney, N.E., Tempelman, R.J., Ernst, C.W., 2004. Pharmacological zinc and phytase supplementation enhance metallothionein mRNA abundance and protein concentration in newly weaned pigs. J. Nutr. 134, 538-544.

Mateos, G.G., Martín, F., Latorre, M.A., Vicente, B., Lazaro, R., 2006. Inclusion of oat hulls in diets for young pigs based on cooked maize or cooked rice. Anim. Sci. 82, 57-63.

McCollum, E.V., Hart, E.B., 1908. On the occurrence of a phytin-splitting enzyme in animal tissue. J. Biol. Chem. 4, 497-500.

McCracken, B.A., Spurlock, M.E., Roos, M.A., Zuckermann, F.A., Gaskins, H.R., 1999. Weaning anorexia may contribute to local inflammation in the piglet small intestine. J. Nutr. 129, 613-619.

Molist, F., Gómez de Segura, A., Gasa, J., Hermes, R.G., Manzanilla, E.G., Anguita, M., Pérez, J.F., 2009a. Effects of the insoluble and soluble dietary fibre on the

physicochemical properties of digesta and the microbial activity in early weaned piglets. Anim. Feed Sci. Technol. 149, 346-53.

Molist, F., Gómez de Segura, A., Perez, J.F., Bhandari, S.K., Krause, D.O., Nyachoti, C.M., 2009b. Effect of wheat bran on the health and performance of weaned pigs challenged with *Escherichia coli* K88+. Livest. Sci. (In the Press).

Molist, F., Ywazaki, M., Gómez de Segura, A., Hermes, R.G., Gasa, J., Pérez, J.F., 2010a. Administration of loperamide and addition of wheat bran to the diets of weaner pigs decrease the incidence of diarrhoea and enhance their gut maturation. Br. J. Nutr. 92, 419-427.

Nyachoti, C.M., Omogbenigun, F.O., Rademacher, M., Blank, G., 2006. Performance responses and indicators of gastrointestinal health in early-weaned pigs fed low-protein amino acid-supplemented diets. J. Anim. Sci. 84, 125-134.

O'Dell, B. L. and J. E. Savage. 1960. Effect of phytic acid on zinc availability. Froc. Soc. Exp. Biol. Med.103, 304.

Ofek, I., Goldhar, J., Sharon, N., 1996. Anti-Escherichia coli adhesin activity of cranberry and blueberry juices. Adv. Exp. Med. Biol. 408, 179-183.

Ou, D., Li, D., Cao, Y., Li, X., Yin, J., Qiao, S., Wu, G., 2007. Dietary supplementation with zinc oxide decreases expression of the stem cell factor in the small intestine of weanling pigs. J. Nutr. Biol. 18, 820-826.

Oufir, L.E., Barry, J.L., Flourie, B., Cherbut, C., Cloarec, D., Bornet, F., Galmiche, J.P., 2000. Relationships between transit time in man and in vitro fermentation of dietary fiber by fecal bacteria. Eur. J. Clin. Nutr. 54, 603-609.

Pierce, K.M., Callan, J.J., McCarthy, P., O'Doherty, J.V., 2007. The interaction between lactose level and crude protein concentration on piglet post-weaning performance, nitrogen metabolism, selected faecal microbial populations and faecal volatile fatty acid concentrations. Anim. Feed Sci. Technol. 132, 267-282.

Ralet, M.C., Faulds, C.B., Williamson, G., Thibault, J.F., 1990. Influence of extrusion-cooking on the physicochemical properties of wheat bran. Carbohydr. Res. 263, 257-269.

Revy, P.S., Jondreville, C., Dourmad, J.Y., Nys, Y., 2005. Assessment of dietary zinc requirement of weaned piglets fed diets with or without microbial phytase. J. Anim. Physiol. Anim. Nutr. 90, 50-59.

Sharon, N., Lis, H., 1989. Lectins as cell recognition molecules. Science 246, 227-234.

Spring, P., Wenk, C., Dawson, K.A., Newman, K.E., 2000. The effects of dietary mannanoligosaccharides on cecal parameters and the concentrations of enteric bacteria in the ceca of salmonella-challenged broiler chicks. Poult. Sci. 79, 205-211.

The Council of the European Communities, 1986. Council Directive 86/609/EEC of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of Animals used for Experimental and Scientific purposes. Available: http://europa.eu.int/comm/environment/chemicals/lab_animals/legislation_en.htm Accessed Apr. 25, 2006.

Yin, J., Li, X., Li, D., Yue, T., Fang, Q., Ni, J., Zhou, X., Wu, G., 2009. Dietary supplementation with zinc oxide stimulates ghrelin secretion from the stomach of young pigs. J. Nutr. Biochem. 20, 783-790.

CHAPTER 8

General Discussion

The weaning transition is a complex period during which the piglets have to cope with different social, environmental and dietetic stresses that usually lead to the presence of diarrhoea and low animal performance. The most effective way which has been applied to prevent the problem has been the inclusion of subtherapeutic doses of antibiotics in feed. However, the emergence of antibiotic-resistant bacteria in human medicine has resulted in pressure to remove antibiotics from animal feeding (Stein and Kil, 2006). Various nutritional approaches for optimising the weaning transition and minimising enteric diseases have been tested, such as organic acids (Partanen and Mroz, 1999), prebiotics or probiotics (Zimmerman et al., 2001), symbiotics (Estrada et al., 2001), plant extracts (Manzanilla et al., 2004) or fibrous ingredients (Bikker et al., 2006). The case of fibre ingredients in the post-weaning diet still remains controversial. The greater discrepancies focus on the amount and source of fibre that can be introduced into the post-weaning diet to obtain beneficial results on growth as well as in the intestinal health of the animals. The present work has been to focus on the study of the effects of including WB and SBP on the performance, microbial population and health status of the animals.

8.1. The likely role of fibrous ingredients in the post-weaning diet

For many years nutritionists have not incorporated fibrous ingredients in the post-weaning diets because of the known negative effects of fibre inclusion on feed intake and nutrient digestibility (Eggum, 1995). However, there is growing evidence that the incorporation of fibrous ingredients in the diet may improve the intestinal function by changing the physicochemical properties of digesta and microbial activity and population (Freire et al., 2000; Bikker et al., 2006). These effects may be more significant in early weaning piglets due to the immaturity of the intestinal tract. The inclusion of fibre in the diet can facilitate the establishment of beneficial microbiota in the intestinal tract (Van Nevel et al., 2006).

Therefore, if we accept this fact, the discussion revolves around which type of fibre and the amount to be introduced into the diet. To answer these questions it is necessary to study the whole the process that happens in the post-weaning

period of pigs, including the anorexia that occurs as well as the immaturity of their intestinal tract and their immune system.

There is conflicting evidence as to whether the type of NSP (iNSP or sNSP) exerts beneficial or detrimental influence on pig health. Highly fermentable sources of sNSP are thought to undergo virtually complete fermentation in the large intestine unlike iNSP which tend to be less fermentable (Longland et al., 1994). It has been suggested that increasing the diet with soluble fibre may increase the viscosity of the intestinal digesta and enhance the proliferation of pathogenic bacteria (Mc. Donald et al., 2001). In contrast, feeding animals with fermentable NSP may reinforce commensal microbiota in the hindgut by the stimulation of carbohydrate fermentation (Bach Knudsen et al., 1991). Including NSP in the starter diet therefore might not only prevent digestive disturbances in the weaner pig, but also contribute to the adaptation of the digestive function of the large intestine. The effects of sNSP may vary according to whether or not sNSP increase the viscosity of the intestinal digesta (Wellock et al., 2007).

The first experiment of this present work (Molist et al., 2009a) was conducted in order to evaluate the type of fibre source included in the diet on the performance and health status of the animals. Therefore, the experimental diets were designed to deliver a range in the amount (from 77 to 96g NDF/kg or 102 to 145g NSP/kg) and type (insoluble and soluble) of NSP. WB was chosen because of its higher proportion of iNSP, whereas SBP contains a higher proportion of sNSP. Results showed the low fermentation of fibre in the first 10 days after weaning was due to the immaturity of the piglet's intestinal tract. The main increases in fermentation were observed between days 10 and 15 after weaning. WB promoted increases on the average daily gain (ADG) of the animals and decreases on the enterobacteria and on coliform shedding. We discussed the likely contribution to theses changes of the effect observed of WB in the physicochemical properties of digesta (ie. an increase in the WRC). The positive results found in the first experiment regarding animal performance and health status encouraged us to choose WB as the fibre source to be used in post-weaning diets.

In the following study we evaluated the optimal level of WB to be included in the diet for early weaned pigs (the results are not shown in this thesis). For this

reason an *in-vivo* experiment with weaning pigs was conducted (Molist et al., 2008) with three dietary treatments: a typical standard post-weaning diet based on corn, barley and soya bean protein, and two fibrous enriched diets including 4 and 8% of WB (WB-4 and WB-8). Feeding animals with WB-4 resulted in a higher feed intake and with a reduction of the enteroccoci and coliform population in the faeces compared to the other experimental diets. Therefore, we chose a level of 4% WB for the rest of the studies in order to achieve the same beneficial effects on the intestinal tract and do not penalize the feed consumption attributed to the higher level of fibre in the diet. The literature shows similar studies with a wide range of levels of DF from 20% (Freire et al., 2000) to a similar level to the one that we use (Montagne et al., 2004) in post-weaning piglets.

The following trials (Molist et al., 2009b; 2010a,b) were designed to confirm the results obtained in the first trial and to elucidate the mechanisms by which WB affect the composition and activity of the intestinal microbiota with the aim of improving gut health.

8.2. Effect of wheat bran on pig performance and nutrient digestibility

Although it was not a main objective of our work, because the low number of replicates in the studies, we measured the effect of fibre inclusion in the performance of post-weaning piglets in three (Molist et al., 2009a, 2010a,b) of the four trials (Table 1). In general, the inclusion of WB in the diet did not negatively affect animal performance compared to a standard cereal based post-weaning diet. On the other hand, the inclusion of WB in the diet in the first trial (Molist et al., 2009a) increased the average daily feed intake (ADFI) in the first week after weaning which resulted in a tendency to lower weight loss of the animals. These results are in good accordance with other works (Hogberg and Lindberg, 2006; Mateos et al., 2006). The latter of these authors suggested that young piglets may have a minimum requirement of fibre for the correct functioning of the digestive tract. However, we should not exclude the possibility that the effects on growth performance of fibre could be due, at least in part, to the increased weight of the gut contents (Pond et al., 1986).

As expected, pigs fed on the WB diet showed a reduction in the total tract digestibility of organic matter (OM) (Molist et al., 2009a, 2010a) and of crude protein (CP) (Molist et al., 2010a). In practice, fibrous diet components dilute the nutrient in feed because the NSP fraction is digested to a lower extent than other fractions such as those of starch, CP, or fat (Morales et al., 2002; Bach Knudsen et al., 2005). Furthermore, the relatively low digestibility of fractions of cell walls of WB diets might be explained by their higher level of lignin (Graham et al., 1986). On the other hand, the increase in OM digestibility with the inclusion of SBP in feed (Molist et al., 2009a) may be a consequence of its high level of fermentable pectins (Longland et al., 1994).

After evaluating the effect of incorporating WB on the growth of the animals in the post-weaning period; we can conclude that the addition of moderate levels of an insoluble fibre source in the diet does not compromise the performance of piglets as compared with a standard diet without in-feed growth promoters.

8.3. Effect of wheat bran on the composition of the intestinal microbiota

The inclusion of WB in the diet resulted in various experiments on a decrease of the enterobacteria (Molist et al., 2009a, 2010a), coliform (Molist et al., 2010b) and *E. coli* (Molist et al., 2009b, 2010b) population in the intestinal tract, indicating a beneficial shift in the composition of the microbial population. These results agree with the results reported by other authors with similar levels of insoluble fibre, such as WB, purified cellulose or pea hulls incorporated into the diet (Van Nevel et al., 2006; Wellock et al., 2007; Becker et al., 2009).

During the different experiments we studied whether the effect of the WB inclusion on the intestinal microbiota could be due to:

8.3.1. Effect of the wheat bran particle size

The incorporation of WB in the post-weaning diet reduced the *E. coli* population in the faeces and the adhesion of *E. coli* K88 to the ileum mucosa, reducing the incidence of diarrhoea (Molist et al., 2009b). This effect was more pronounced when WB was incorporated in the diet in coarse particles. These results

are in good agreement with Mikkelsen et al. (2004) that suggested that a coarse diet modifies the microbiota in the GIT, with a reduction in the gastric population of enterobacteria mainly by changing the environment in the proximal GIT. Moreover, the incorporation of WB in coarse particles reduced the microbial richness in ileal digesta to a similar level as that of the diet supplemented with antibiotics, whereas WB included as fine particles increased microbial diversity. These results suggests that the particle size of WB is an important factor with regard to its effect on microbiota, probably associated to changes in the physicochemical properties of digesta or due to the physical effect on the intestinal epithelium of larger particles. As well as interesting, these results are very positive for the animals because in the small intestine either ileum or jejunum is where *E. coli* can attach to the mucosa, proliferating to cause post-weaning diarrhoea (Jones and Rutter, 1972).

8.3.2. Anti-adhesion activity of wheat bran

Another mechanism that could explain the reduction of the *E. coli* adhesion to the ileum mucosa when animals consumed the WB diet could be related to the ability of WB to bind to *E. coli* (Molist et al., 2010b). In the final trial the supernatant obtained after sonicating and centrifuging a suspension of 4% WB showed a higher binding to *E. coli* compared to other fibre ingredients such as SBP, pea and soya bean hulls, or barley straw. These results agree with the results obtained in the experimental infection of *E. coli* K88 (Molist et al., 2009b). This effect could be explained by the NSP content of the WB. This ingredient contains iNSP (Ralet et al., 1990) mainly arabinoxylan, cellulose and ß-glucan, but also minute levels of glucomannans (Mares and Stone, 1973) and arabinogalactans (Fincher et al., 1974) originating from the aleurone and endosperm cells. It might be speculated that WB may form a matrix in the gut where fimbriated *E. coli* could be captured and grown.

8.3.3. Effect of wheat bran on the modification of the intestinal environment

The incorporation of WB in the diet resulted in a pronounced increase in the concentration of SCFA (Molist et al., 2009a, 2010a,b). The increases on the SCFA concentration with the NSP diets could be associated with a higher WRC of digesta

(Auffret et al., 1993) as observed in the present study. The WRC is correlated to the amount of DF, but is also dependent on the composition and structural features of fibre (Bertin et al., 1988). At the same time, WRC has been used as a predictor of the degradability of fibres (McBurney et al., 1987; Auffret et al., 1993). The higher the WRC, the more intensive the extent of fibre fermentation. Several studies have shown that effects on the reduction in enterobacteria, coliform and *E. coli* numbers were related to the SCFA concentrations (Burnett and Hanna, 1963; Mathew et al., 1998). The SCFA are believed to play an important role in reducing the numbers of enterobacteria related to the undissociated form of lactic acid and other SCFA (Russell and Diez-Gonzalez, 1998). Moreover, fibre inclusion may also improve the health status of the animals by increasing the level of butyric acid in the digesta. Butyrate is considered an important metabolite because it is the principal oxidative fuel for the colonocytes and may have beneficial trophic effects on the inflamed caeco-colonic mucosa (Oufir et al., 2000). In three of the four experiments, WB inclusion increased the butyric acid concentration in the colon (Molist et al., 2009a) and in the faeces (Molist et al., 2010a,b) of weaned pigs. This high level of butyric acid found in the digesta may be generated by small quantities of endosperm that escape from the digestion in the small intestine and fermented in the large intestine (Freire et al., 2000).

Finally, another benefit for gut health associated to WB inclusion is the reduction of protein fermentation and the concentration of branched chain fatty acids in the digesta (Molist et al., 2010a). Addition of fibre in the diet decreases the need of the animals to ferment protein, reducing the formation of biogenic amines and the incidence of post-weaning diarrhoea (Pluske et al., 2003). Awati et al. (2006) also reported that the inclusion of fermentable CH in weanling diets reduces the protein fermentation along the GIT. Therefore it can be concluded that inclusion of iNSP in the diet may not only prevent digestive disturbances in the weaner pig but also contribute to the adaptation of the digestive function of the large intestine.

8.4. Negative interaction between wheat bran and zinc oxide

The simultaneous incorporation of high doses of ZnO with moderate levels of WB (Molist el al., 2010b) gave us the surprising result of an interaction between

ZnO and the phytate of WB on the intestinal microbiota. Until now, no literature has been available regarding the negative interaction between the ZnO and the phytate content on the microbial activity and composition in the GIT. As it is shown in the results of the last trial (Molist et al., 2010b) this interaction affected the effect of both ingredients. On one hand, it deactivated the ability of ZnO to act as antimicrobial agent and on the other hand also decreased the SCFA production related to the WB inclusion. Champagne and Fisher (1990) suggest that phytic acid (PA), primarily found in the pericarp of the cereal grains, may form a rather stable complex with bivalent cations, such as the Cu^{2+} and Zn^{2+}, that precipitates at Zn:phytate molar ratios of 3.5 to 4:1. These complexes are known not only to affect the Zn bioavailability, but also to decrease the availability of the phytate for the hydrolysis by phytase (Augspurger et al., 2004). Therefore some efforts have been carried out to find strategies to diminish the formation of phytates complexes. Some authors have studied procedures to degrade phytates as a means to increase the bioavailability of Zn and other cations in the diet and therefore increase the nutritional value of the meal (Bobilya et al., 1991). In the same way, acidification of the feed by fermentation (Hirabayashi et al., 1998) or by addition of organic acids such as acetic acid (Valencia and Chavez, 2002) have resulted in an enhancement of Zn absorption. However, the most common strategy in monogastric animals is to include exogenous phytase in the feed to remove phytate and increase the cations availability. This is supported by the results obtained in the *in-vitro* study (Molist et al., 2010b), in which the removal of the phytic acid of the WB with the addition of phytase to the mixture resulted in the ZnO inhibition of the growth of the *E. coli* regardless of the fimbrial type. Taking into account that the post-weaning pig diets are based on cereals, we suggest incorporating exogenous phytase into the diet or to find new ways (organic rather than inorganic forms) of incorporating Zn into the diet in order to increase its bioavailability for the animal and so reduce its excretion in the feaces.

CHAPTER 9

Conclusions

The results obtained in this thesis allow us to conclude that in our experimental conditions:

1. Inclusion of moderate levels of wheat bran and sugar beet pulp (40 – 80 g/kg) in post-weaning diets may increase the voluntary feed intake and performance of the animals despite the lower digestibility of fibrous ingredients. This effect indicates the positive effect of fibrous diets to promote a healthy digestive tract.

2. Inclusion of coarse wheat bran in the diet of early weaned piglets modifies the physicochemical properties and the microbial fermentation of intestinal digesta, which may be referred as positive for the intestinal tract. Wheat bran increases water retention capacity and butyric acid concentration and enhance the microbial fermentation in the intestinal digesta.

3. The introduction of coarse wheat bran in the diet of post-weaning piglets reduces the counts of enterobacteria and coliform bacteria in the intestinal digesta, and specifically the counts of *E. coli* K88 attached to the ileum mucosa. Our results confirm that this effect may be due to, at least in part, to mechanisms of blockage of the bacterial adhesion.

4. The simultaneous incorporation of wheat bran and zinc oxide in the diet promotes a negative interaction between ingredients which modifies its individual results on the intestinal microbiota and fermentation. Some compounds of wheat bran, such as phytate, may be involved on the reduction of the antimicrobial activity of zinc oxide, which allow as to recommend the incorporation of phytases in early weaning diets.

CHAPTER 10

References

Allen, V.M., Fernandez, F., Hinton, M.H., 1997. Evaluation of the influence of supplementing the diet with mannose or palm kernel meal on Salmonella colonisation in poultry. Br. Poult. Sci. 38, 485-488.

Anguita, M., 2006. Physicochemical characteristics of vegetal feedstuffs in growing pig diets: Influence of the carbohydrate fraction on the energy value and the gastrointestinal tract environment. PhD Diss. Universitat Autònoma de Barcelona, Bellaterra, Spain.

Auffret, A., Barry, J.L., Thibault, J.F., 1993. Effect of chemical treatments of sugar-beet fiber on their physicochemicals properties and on their in vitro fermentation. J. Sci. Food Agric. 61, 195-203.

Augspurger, N.R., Spencer, J.D., Webel, D.M., Baker, D.H., 2004. Pharmacological zinc levels reduce the phosphorus-releasing efficacy of phytase in young pigs and chickens. J. Anim. Sci. 82, 1732-1739.

Awati, A., Williams, B.A., Bosch, M.W., Gerrits, W.J.J., Verstegen, M.W.A., 2006. Effect of inclusion of fermentable carbohydrates in the diet on fermentation end-product profile in feces of weanling piglets. J. Anim. Sci. 84, 2133-2140.

Bach Knudsen, K.E., Jensen, B.B., Andersen, J.O., Hansen, I., 1991. Gastrointestinal implications in pigs of wheat and oat fractions. 2. Microbial activity in the gastrointestinal tract. Br. J. Nutr. 65, 233-248.

Bach Knudsen, K.E., Serena, A., Kjaer, A.K.B., Jorgensen, H., Engberg, R., 2005. Rye bread enhances the production and plasma concentration of butyrate but not the plasma concentrations of glucose and insulin in pigs. J. Nutr. 135, 1696-1704.

Becker, P.M., Van Wikselaar, P.G., Jansman, A.J.M., Van der Meulen, J., 2009. Pea dietary fiber for adhesion and excretion of enterotoxigenic *E. coli* K88 to prevent intestinal colonization. J. Anim. Sci. 87, 172.

Bedford, M.R., Classen, H.L., 1992. Reduction of intestinal viscosity through manipulation of dietary and pentosane concentration is effected through changes in the carbohydrate composition of the intestinal aqueous phase results in improved growth rate and food conversion efficiency of broiler chicks. J. Nutr. 122, 560-569.

Bergman, E.N., 1990. Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. Physiol. Rev. 70, 567-590.

Bertin, C., Rouau, X., Thibault, J.F., 1988. Structure and properties of sugar beet fibres. J. Sci. Food Agric. 44, 15-29.

Bikker, P., Dirkzwager, A., Fledderus, J., Trevisi, P., Le Huërou-Luron, I., Lallès, J.P., Awati, A., 2006. The effect of dietary protein and fermentable carbohydrates levels on growth performance and intestinal characteristics in newly weaned piglets. J. Anim. Sci. 84, 3337-3345.

Bindelle, J., Leterme, P., Buldgen, A., 2008. Nutritional and environmental consequences of dietary fibre in pig nutrition: a review. Biotechnol. Agronom. Soc. Environ. 12, 69-80.

Blomberg, L., Krivan, H.C., Cohen, P.S., Conway, P.L., 1993. Piglet ileal mucus contains protein and glycolipid (galactosylceramide) receptors specific for *Escherichia coli* K88 fimbriae. Infect. Immun. 61, 2526-2531.

Bobilya, D.J., Ellersieck, M.R., Gordon, D.T., Veum, T.L., 1991. Bioavailabilities of zinc from non-fat dry milk, low-fat plain yogurt, and soy flour in diets fed to neonatal pigs. J. Agric. Food Chem. 39, 1246-1251.

Bogovic Matijasic, B., Stojkovic, S., Rogeli, I., 2006. Survival and *in vivo* adhesion isolates *Lactobacillus gasseri* LF221 and K7 in weaned piglets and their effects on coliforms, clostridia and lactobacilli viable counts in faeces and mucosa. J. Dairy Res. 73, 417-422.

Boudry, G., Péron, V., Le Huërou-Luron, I., Lallès, J.P., Sève, B., 2004. Weaning induces both transient and long-lasting modifications of absorptive, secretory, and barrier properties of piglet intestine. J. Nutr. 134, 2256-2262.

Brooks, P.H., Moran, C.A., Beal, J.D., Demeckova, V., Campbell, A., 2001. Liquid feeding for the young piglet, in: Varley, M.A., Wiseman, J. (Eds.), The weaner pig: Nutrition and Management. CAB International, Wallingford, UK, pp. 153-178.

Brunsgaard, G., 1998. Effects of cereal type and feed particle size on morphological characteristics, epithelial cell proliferation, and lectin binding patterns in the large intestine of pigs. J. Anim. Sci. 76, 2787-2798.

Burnett, G.S., Hanna, J., 1963. Effect of dietary calcium lactate and lactic acid on fecal *Escherichia coli* counts in pigs. Nature 197, 815.

Burrin, D.G., Wester, T.J., Davis, T.A., Heath, J.P., McAvoy, S., Skottner, A., 1995. Oral insulin-like growth factor 1 (IGF-1) increases small intestinal growth in formula-fed neonatal pigs, in: Proc. of the 5th Int. IGF-1 Symposium Florida, USA, pp. 15.

Canibe, N., Bach Knudsen, K.E., 2002. Degradation and physicochemical changes of barley and pea fibre along the gastrointestinal tract of pigs. J. Sci. Food Agric. 82, 27-39.

Castillo, M., 2006. Development of gut microbiota in the pig: modulation of bacterial communities by different feeding strategies. PhD Diss. Universitat Autònoma de Barcelona, Bellaterra, Spain.

Castillo, M., Martín-Orúe, S.M, Nofrarías, M., Manzanilla, E.G., Gasa, J., 2007a. Changes in caecal microbiota and mucosal morphology of weaned pigs. Vet. Microbiol. 124, 239-247.

Castillo, M., Martín-Orúe, S.M., Anguita, M., Pérez, J.F., Gasa, J., 2007b. Adaptation of gut microflora to corn physical structure and different types of dietary fibre. Livest. Sci. 109, 149-152.

Champagne, E.T., Fisher, M.S., 1990. Binding differences of Zn(II) and Cu(II) ions with phytate. J. Inorg. Biochem. 38, 217-223.

Claus, R., Günthner, D., Letzgub, H., 2007. Effects of feeding fat-coated butyrate on mucosal morphology and function in the small intestine of the pig. J. Anim. Physiol. Anim. Nutr. 91, 312-318.

Collado, M.C., Grześkowiak, L., Salminen, S., 2007. Probiotic strains and their combination inhibit *in vitro* adhesion of pathogens to pig intestinal mucosa. Cur. Microbiol. 55, 260-265.

Conway, P.L., Welin, A., Cohen, P., 1990. Presence of K88-specific receptors in porcine ileal mucus is age dependant. Infect. Immun. 58, 3178–3182.

Conway, P.L., 1994. Function and regulation of the gastrointestinal microbiota of the pig. EAPP publication. 2, 231-240.

Cummings J.H., Englyst, H.N., 1987. Fermentation in the human large intestine and the available substrates. Am. J. Clin. Nutr. 45, 1243S-1255S.

Dantzer, R., Mormede, P., 1983. Stress in farm animals: a need for reevaluation. J. Anim. Sci. 57, 6-18.

Dean, E.A., 1990. Comparison of receptors for 987P pili of enterotoxigenic *Escherichia coli* in the small intestines of neonatal and older pig. Infect. Immun. 58, 4030-4035.

Dean-Nystrom, E.A., Casey, T.A., Scneider, R.A., Nagy, B., 1993. A monoclonal antibody identifies 2134P fimbriae as adhesins on enterotoxigenic *Escherichia coli* isolated from postweaning pigs. Vet. Microbiol. 37, 101-114.

Dean-Nystrom, E.A., Samuel, J.E., 1994. Age-related resistance to 987P fimbria-mediated colonization correlates with specific glycolipid receptors in intestinal mucus in swine. Infect. Immun. 62, 4789-4794.

Deprez, P., Deroose, P., Van den Hende, C., Muylle, E., Oyaert, W., 1987. Liquid versus dry feeding in weaned piglets: The influence on small intestinal morphology. J. Vet. Med. Biol. 34, 254-259.

Eggum, B.O., 1995. The influence of dietary fibre on protein digestion and utilization in monogastrics. Arch. Anim. Nutr. 48, 89-95.

Eliasson, A.C., Gudmundsson, M., 1996. Starch: Physicochemical and functional aspects, in: Eliasson A.C. (Eds.), Carbohydrates in Food. Marcel Dekker, Lund, Sweden, pp. 413.

Erickson, A.K., Baker, D.R., Bosworth, B.T., Casey, T.A., Benfield, D.A., Francis, D.H., 1994. Characterization of porcine intestinal receptors for the K88ac fimbrial adhesion of *Escherichia coli* as mucin-type sialoglycoproteins. Infect. Immun. 62, 5404-5410.

Estrada, A., Drew, M.D., Van Kessel, A., 2001. Effect of the dietary supplementation of fructooligosaccarides and *Bifidobacterium longum* to early weaned pigs on performance and fecal bacterial populations. Can. J. Anim. Sci. 81, 141-148.

Ewing, W.N., Cole, D.J.A., 1994. The microbiology of the gastrointestinal tract, in: Ewing, W.N., Cole, D.J.A. (Eds.), The living gut: An introduction to microorganisms in nutrition. Context, Ireland, UK, pp. 45-65.

Fadel, J.G., Newman, C.W., Newman, R.K., Graham, H., 1988. Effects of extrusion cooking of barley on ileal and fecal digestibilities of dietary-components in pigs. Can. J. Anim. Sci. 68, 891-897.

Fairbrother, J.M., 1992. Enteric colibacillosis, in: Leman, D.E., Straw, B.E., Mengeling, W.L., D'Allaire, S.D., Taylor, D.J. (Eds.), Disease of Swine. Iowa State University Press, Ames, Iowa, pp. 489-497.

Ferguson, L.R, Harris, J.P., 1997. Particle size of wheat bran in relation to colonic function in rats. Lebensm.Wiss. Technol. 30, 735-742.

Fernandez, F., Hinton, M., Van Gils, B., 2002. Dietary mannan-oligosaccharides and their effect on chicken caecal microflora in relation to *Salmonella enteritidis* colonization. Avian Pathol. 31, 49-58.

Fincher, G.B., Stone, B.A., 1986. Cell walls and their components in cereal grain technology, in: Pomeranz, Y. (Ed.), Advances in cereal science and technology, AACC. International, St Paul, Minnesota, pp. 207-295.

Fontaine, N., Meslin, J.C., Dore, J., 1998. Selective in vitro degradation of the sialylated fraction of germ-free rat mucins by the caecal flora of the rat. Reprod. Nutr. Dev. 38, 289-296.

Forstner, J.F., Fostner, G.G., 1994. Gastrointestinal mucus, in: Johnson L.R. (Ed.), Physiology of the Gastrointestinal Tract. Raven Press, New York, pp. 1255-1283.

Freire, J.P.B., Guerreiro, A.J.G., Cunha, L.F., Aumaitre, A., 2000. Effect of dietary fibre source on total tract digestibility, caecum volatile fatty acids and digestive transit time in the weaned piglet. Anim. Feed Sci. Technol. 87, 71-83.

Fuller, M.F., Reeds, P.J., 1978. Nitrogen cycling in the gut. Ann. Rev. Nutr. 18, 385-411.

Gardner, K.H., Blackwell, J., 1974. The hydrogen bonding in native cellulose. Biochim. Biophys. Acta 343, 232-237.

Garst, A.S., Ball, S.F., Williams, B.L., Wood, C.M., Knight, J.W., Moll, H.D., Aardema, C.H., Gwazdauskas, F.C., 1999. Influence of pig substitution on milk yield, litter weights, and milk composition of machine milked sows. J. Anim. Sci. 77, 1624-1630.

Goodlad, R.A., Wright, N.A., 1983. Effects of addition of kaolin or cellulose to elemental diet on intestinal-cell proliferation in the mouse. Br. J. Nutr. 50, 91-98.

Graham, H., Hesselman, K., Aman, P., 1986. The influence of wheat bran and sugar-beet pulp on the digestibility of dietary components in a cereal based pig diet. J. Nutr. 116, 242-251.

Grange, P.A., Mouricout, M.A., 1996. Susceptibility of infant mice to F5 (K99) *E. coli* infection: Differences in glycosyltransferase activities in intestinal mucosa of inbred CBA and DBA/2 strains. Glycoconjugate J. 13, 45-52.

Grange, P.A., Erickson, A.K., Anderson, T.J., Francis, D.H., 1998. Characterization of the carbohydrate moiety of intestinal mucin-type sialoglycoprotein receptors for the K88ac fimbrial adhesion of *Escherichia coli*. Infect. Immun. 65, 1613-1621.

Grange, P.A., Mouricout, M.A., Levery, S.B., Francis, D.H., Erickson, A.K., 2002. Evaluation of receptor binding specificity of *Escherichia coli* K88 (F4) fimbrial adhesion variants using porcine serum transferring and glycosphingolipids as model receptors. Infect. Immun. 70, 2336-2343.

Grange, P.A., Parrish, L.A., Erickson, A.K., 2006. Expression of putative *Escherichia coli* heat-labile enterotoxin (LT) receptors on intestinal brush borders from pigs of different ages. Vet. Res. Commun. 30, 57-71.

Greenwood, C.T., 1979. Observation of the structure of the starch granule. Polysaccharides in food, in: Blanshard, J.M.V., Mitchel, R.J. (Eds.), Polysaccharides in food. Butterworth's and Company Ltd., London, pp. 129-138.

Guinee, P.A.M., Veldkamp, J., Jansen, W.H., 1977. Improved Minca medium for the detection of K99 antigen in calf enterotoxigenic strains of *Escherichia coli*. Infect. Immun. 15, 676-678.

Hammes, W.P., Weiss, N., Holzapfel, W., 1991. The genera Lactobacillus and Carnobacterium, in: Bolows, A., Trüper, H.G., Dworkin, M., Harder, W., Schleifer, K.H. (Eds), The Prokaryotes: A Handbook on the Biology of Bacteria:

Ecophysiology, Isolation, Identification, Applications. Springer-Verlag, New York, USA, pp.1535-1594.

Hampson, D.J., 1987. Dietary influences on porcine post-weaning diarrhoea, in: Barnett J.L., Batterham, E.S., Cronin, G.M., Hansen, C., Hemsworth, P.H., Hennessy, D.P., Hughes, P.E., Johnston, N.E., King, R.H. (Eds.), Manipulating Pig Production. Australasian Pig Science Association, Werribee, Victoria, Australia, pp. 202-214.

Hedemann, M.S., Mikkelsen, L.L., Naughton, P.J., Jensen, B.B., 2005. Effect of feed particle size and feed processing on morphological characteristics in the small and large intestine of pigs and on adhesion of *Salmonella enterica* serovar Typhimurium DT12 in the ileum in-vitro. J. Anim. Sci. 83, 1554-1562.

Hedemann, M.S., Eskildsen, M., Laerke, H.N., Pedersen, C., Lindberg, J.E., Laurinen, P., Bach Knudsen, K.E., 2006. Intestinal morphology and enzymatic activity in newly weaned pigs fed contrasting fiber concentrations and fiber properties. J. Anim. Sci. 84, 1375-1386.

Hedemann, M.S., Bach Knudsen, K.E., 2007. Resistant starch for weaning pigs – Effect on concentration of short chain fatty acids in digesta and intestinal morphology. Livest. Sci. 108, 175-177.

Hermes, R.G., Molist, F., Ywazaki, M., Nofrarías, M., Gómez de Segura, A., Gasa, J., Pérez, J.F., 2009. Effect of dietary level of protein and fiber on the productive performance and health status of piglets. J. Anim. Sci. 87, 3569-3577.

Hentges, D.J., 1986. The protective function of the indigenous intestinal flora. Ped. Inf. Dis. 5, 17-20.

Herkelman, K.L., Rodhouse, S.L., Veum, T.L., Ellersieck, M.R., 1990. Effect of extrusion on the ileal and fecal digestibilities of lysine in yellow corn in diets for young pigs. J. Anim. Sci. 68, 2414-2424.

Hirabayashi, M., Matsui, T., Yano, H., 1998. Fermentation of soybean meal with *Aspergillus usamii* improves zinc availability in rats. Biol. Trace Elem. Res. 61, 227-234.

Hizukuri, S., 1996. Starch: analytical aspects, in: Eliasson, A.C. (Ed.), Carbohydrates in Food. Marcel Dekker, Lund, Sweden, pp. 347-429.

Hogberg, A., Lindberg, J.E., 2006. The effect of level and type of cereal non-starch polysaccharides on the performance, nutrient utilization and gut environment of pigs around weaning. Anim. Feed Sci. Technol. 127, 200-219.

Hopwood, D.E., Hampson, D.J., 2003. Interactions between the intestinal microflora, diet and diarrhoea, and their influences on piglet health in the immediate post-weaning period, in: Pluske, J.R, LeDividich, J., Verstegen, M.W.A. (Eds.), Weaning the pig. Concepts and Consequences. Wageningen Academic Publishers, The Netherlands.

Horosová, K., Bujnáková, D., Kmet, V., 2006. Effect of lactobacilli on *E. coli* adhesion to Caco-2 cells in vitro. Folia Microbiol. 51, 281-282.

Hurley, W.L., Wang, H., Bryson, J.M., Shennan, D.B., 2000. Lysine uptake by mammary gland tissue from lactating sows. J. Anim. Sci. 78, 391-395.

Idänpään-Heikkilä, I., Simon, P.M., Zopf, D., Vullo, T., Cahill, P., Sokol, K. Tuomanen, E., 1997. Oligosaccharides interfere with the establishment and progression of experimental pneumococcal pneumonia. J. Infect. Dis. 176, 704-712.

Inoue, R., Tsukahara, T., Nakanishi, N., Ushida, U., 2005. Development of the intestinal microbiota in the piglet. J. Gen. Appl. Microbiol. 51, 257-265.

Isaacson, R.E., Fusco, P.C., Brinton, C.C., Moon, H.W., 1978. In vitro adhesion of *Escherichia coli* to porcine small intestinal epithelial cells: pili as adhesive factors. Infect. Immun. 21, 392-397.

Jackson, J.R., Hurley, W.L., Easter, R.A., Jensen, A.H., Odle, J., 1995. Effects of induced or delayed parturition and supplemental dietary fat on colostrum and milk composition in sows. J. Anim. Sci. 73, 1906-1913.

Jensen, B.B., Agergaard, N., Hansen, L.L., Mikkelsen, L.L., Jensen, M.T., Laue, A., 1998. Effect of liquid feed on microbial production of skatole in the hind gut, skatole absorption to portal vein blood and skatole deposition in back fat, in:

Jensen W.K. (Ed.), Skatole and Boar taint. Danish Meat Research Institute, Roskilde, Denmark.

Jiménez, E., Fernández, L., Marín, M.L., Martín, R., Odriozola, J.M., Nueno-Palop, C., Narbad, A., Olivares, M., Xaus, J., Rodríguez, J.M. 2005. Isolation of commensal bacteria from umbilical chord blood of healthy neonates born by caesarean section. Cur. Microbiol. 51, 270-274.

Jin, L., Reynolds, L.P., Redmer, D.A., Caton, J.S., Crenshaw, J.D., 1994. Effects of dietary fiber on intestinal growth, cell proliferation, and morphology in growing pigs. J. Anim. Sci. 72, 2270-2278.

Jin, L.Z., Baidoo, S.K., Marquardt, R.R., Frohlich, A.A., 1998. In vitro inhibition of adhesion of enterotoxigenic *Escherichia coli* K88 to piglet intestinal mucus by egg-yolk antibodies. FEMS Immunol. Med. Microbiol. 21, 313-321.

Jin, L.Z., Zhao, X., 2000. Intestinal receptors for adhesive fimbriae of enterotoxigenic *Escherichia coli* (ETEC) K88 in swine: a review. Appl. Microbiol. Biotechnol. 54, 311-318.

Johansen, H.N., Bach Knudsen, K.E., Sandstrom, B., Skjoth, F., 1996. Effects of varying content of soluble dietary fibre from wheat flour and oat milling fractions on gastric emptying in pigs. Br. J. Nutr. 75, 339-351.

Jones, G.W., Rutter, J.M., 1972. Role of the K88 antigen in the pathogenesis of neonatal diarrhoea caused by *Escherichia coli* in piglets. Infect. Immun. 6, 918-927.

Jorgensen, H., Zhao, X.Q., Eggum, B.O., 1996. The influence of dietary fibre and environmental temperature on the development of the gastrointestinal tract, digestibility, degree of fermentation in the hind-gut and energy metrabolism in pigs. Br. J. Nutr. 75, 365-378.

Kahane, I., Ofek, I., 1996. Toward anti-adhesion therapy for microbial diseases. Adv. Exp. Med. Biol. 408, 1-288.

Kelly, D., Smyth, J.A., McCracken, K.J., 1991. Digestive development in the early-weaned pig. II. Effect of level of food intake on digestive enzyme activity during the immediate post-weaning period. Br. J. Nutr. 65, 181-188.

Kelly, C.G., Younson, J.S., 2000. Anti-adhesive strategies in the prevention of infectious disease at mucosal surfaces. Expert. Opin. Investig. Drugs 9, 1711-1721.

Khan, A.S., Schifferli, D.M., 1994. A minor 987P protein different from the structural fimbrial subunit is the adhesion. Infect. Immun. 62, 4223-4243.

Khan, A.S., Johnston, N.C., Goldfine, H., Schifferli, D.M., 1996. Porcine 987P glycolipid receptors on intestinal brush borders and their cognate bacterial ligands. Infect. Immun. 64, 3688-3693.

Kien, C.L., Blauwiekel, R., Bunn, J.Y., Jetton, T.L., Frankel, W.L., Holst, J.J., 2007. Cecal infusion of butyrate increases intestinal cell proliferation in piglets. J. Nutr. 137, 916-922.

Kiers, J.L., Nout, M.J.R., Rombouts, F.M., Nabuurs, M.J.A., Van der Meulen, J., 2002. Inhibition of adhesion of enterotoxigenic *Escherichia coli* K88 by soya bean tempe. Lett. Appl. Microbiol. 35, 311-315.

Kim, J.C., Mullan, B.P., Hampson, D.J., Pluske, J.R., 2008. Addition of oat hulls to an extruded rice-based diet for weaner pigs ameliorates the incidence of diarrhoea and reduces indices of protein fermentation in the gastrointestinal tract. Br. J. Nutr. 99, 1217-1225.

Klessen, B., Blaut, M., 2005. Modulation of gut mucosal biofilms. Br. J. Nutr. 93, S35-S40.

Konstantinov, S.R., Favier, C.F., Zhu, W.Y., Williams, B.A., Klub, K., Souggrant, W.B., De Vos, W.M., Akkermans, A.D.L., Smidt, H., 2004. Microbial diversity studies on the porcine gastrointestinal ecosystem during weaning transition. Anim. Res. 53, 317-324.

Kuniak, L., Marchessault, R.H., 1972. Study of the cross-linking reaction between epichlorohydrin and starch. Starch/Stärke 24, 110-116.

Kyogashima, M., Ginsburg, V., Krivan, H.C., 1989. *Escherichia coli* K99 binds to N-glycolylsialoparagloboside and N-glycolyl-GM3 found in piglet small intestine. Arch. Biochem. Biophys. 270, 391-397.

Ladd, C.O., Huot, R.L., Thrivikraman, K.V., Nemeroff, C.B., Meaney, M.J., Plotsky, P.M., 2000. Long-term behavioural and neuroendocrine adaptations to adverse early experience, in: Mayer, E.A., Saper, C.B., (Eds.), Progress in brain research: the biological basis for mind body interactions. Elsevier, Amsterdam, pp. 81-103.

Laerke, H.N., Hedemann, M.S., Bach Knudsen, K.E., Laurinen, P., Lindberg, J.E., Pedersen, C., 2007. Association between butyrate and short-chain fatty acid concentrations in gut contents and faeces in weaning piglets. Livest. Sci. 108, 163-166.

Lallès, J.P., Salmon, H., Bakker, N.P.M., Tolman, G.H., 1993. Effects of dietary antigens on health, performance and immune system of calves and piglets, in: Souffrant, W.B., Hagemeister, H. (Eds), VIth International Symposium on Digestive Physiology in Pigs. EAAP Publication nº 80. Forchungsinstitut für die Biologie landwirtschahtlicher Nutztiere (FBN), Dummerstorf, Germany, pp. 295-307.

Lallès, J.P., Boudry, G., Favier, C., Le Floc'h, N., Luron, I., Montagne, L., Oswald, I.P., Pié, S., Piel, C., Sève, B., 2004. Gut function and dysfunction in young pigs: physiology. Anim. Res. 53, 301-316.

Lallès, J.P., Bosi, P., Smidt, H., Stokes, C.R., 2007. Weaning - A challenge to gut physiologists. Livest. Sci. 108, 82-93.

Le Dividich, J., Sève, B., 2002. Effects of underfeeding during the weaning period on growth, metabolism, and hormonal adjustments in the piglet. Domest. Anim. Endocrinol. 19, 63-74.

Le Goff, G., Noblet, J., 2001. Comparative total tract digestibility of dietary energy and nutrients in growing pigs and adult sows. J. Anim. Sci. 79, 2418-2427.

Lengsfeld, C., Titgemever, F., Faller, G., Hensel, A., 2004. Glycosylated compounds from okra inhibit adhesion of *Helicobacter pylori* to human gastric mucosa. J. Agric. Food Chem. 52, 1495-1503.

Leterme, P., Froidmont, E., Rossi, F., Théwis, A., 1998. The high water-holding capacity of pea inner fibers affects the ileal flow on endogenous aminoacids in pigs. J. Agric. Food Chem. 46, 1927-1934.

Liebler, E.M., Pohlenz, J.F., Whipp, S.C., 1992. Digestive system, in: Leman, A.D., Straw, B.E., Mengeling, W.L., D'Allaire, S., Taylor, D.J. (Eds.), Diseases of Swine. Iowa State University Press, Iowa, pp. 12-20.

Liesnewska, V., Laerke, H.N., Hedemann, M.S., Jensen, B.B., Hojsgaard, S., Pierzynowski, S.G., 2000. Myoelectric activity of gastric antrum in conscious piglets around weaning. Can. J. Anim. Sci. 80, 577-584.

Longland, A.C., Carruthers, J., Low, A.G., 1994. The ability of piglets 4 to 8 weeks old to digest and perform on diets containing two contrasting sources of non-starch polysaccharide. Anim. Prod. 58, 405-410.

Low, A.G., Longland, A.C., 1990. Carbohydrate and dietary fibre digestion in the pig and the possible influence of feed enzymes. Feed Compounder 10, 37-42.

MacFarlane, G.T., Cummings, J.H., 1991. The colonic flora, fermentation and large bowel digestive function, in: Phillips, S.F., Pemberton, J.H., Shorter, R.G. (Eds.), The large intestine: physiology, pathophysiology and disease. Raven Press Ltd., New York, USA, pp. 51-92.

MacFarlane, G.T., McBain, A.J., 1999. The human colonic microflora, in: Gibson, G.R., Roberfroid, M.B. (Eds.), Colonic microflora, nutrition and health. Kluwer Academic Publishers, The Netherlands, pp. 1-25.

MacFarlane, S., MacFarlane, G.T., 2003. Regulation of short-chain fatty acid production. Proc. Nutr. Soc. 62, 67-72.

Mackie R. I., Sghir, A., Gaskins, H.R., 1999. Developmental microbial ecology of the neonatal gastrointestinal tract. Am. J. Clin. Nutr. 69, 1035S-1045S.

Maiorano, R., Jongbloed, A.W., Wagenaars, C.M.F., Van Wikselaar, P.G., Becker, P.M., 2007. Evaluation of plant materials for alternative adhesion of *E. coli* K88 (ETEC) in weaning pigs. J. Anim. Sci. 85(S1).

Manzanilla, E.G., Pérez, J.F., Martin, M., Kamel, C., Baucells, F., Gasa, J., 2004. Effect of plant extracts and formic acid on the intestinal equilibrium of early-weaned pigs. J. Anim. Sci. 82, 3210-3218.

Mares, D.J., Stone, B.A., 1973. Studies on wheat endosperm: Chemical composition and ultrastructure of cells walls. Aust. J. Biol. Sci. 26, 793-812.

Martinez-Puig, D., Morales, J., Borda, E., Piñeiro, C., Chetri, C., 2007. Dietary nucleotides supplementation improves growth performance of early weaned piglets. J. Anim. Sci. 85(S1), 73.

Mason, S.P., Jarvis, S., Lawrence, A.B., 2003. Individual differences in response of piglets to weaning at different ages. Appl. Anim. Behav. Sci. 80, 117-132.

Mateos, G.G., Alcantarilla, A., Latorre, M.A., Lazaro, R., Gomez, E., Laso, N., 2001. Influence of type of cereal and level of fiber on performance of early weaned piglets. J. Anim. Sci. 79,106-107.

Mateos, G.G., Martín, F., Latorre, M.A., Vicente, B., Lazaro, R., 2006. Inclusion of oat hulls in diets for young pigs based on cooked maize or cooked rice. Anim. Sci. 82, 57-63.

Mathew, A.G., Chattin, S.E., Robbins, C.M., Golden, D.A., 1998. Effects of a direct-fed yeast culture on enteric microbial populations, fermentations acids, and performance of weanling pigs. J. Anim. Sci. 76, 2138-2145.

May, T., Mackie, R.I., Fahey, G.C., Cremin, J.C., Garleb, K.A., 1994. Effect of fiber soured on short-chain fatty acid production and on the growth and toxin production by *Clostridium difficile*. Scand. J. Gastroenterol. 29, 916.

McBurney, M.I., Horvath, P.J., Jeraci, J.L., Van Soest, P.J., 1985. Effect of in vitro fermentation using human fecal inoculum on the water-holding capacity of dietary fiber. Br. J. Nutr. 53, 17-24.

McBurney, M.I., Van Soest, P.J., Jeraci, J.L., 1987. Colonic carcinogenesis: the microbial feast or famine mechanism. Nutr. Cancer 10, 23-28.

McCracken, B.A., Spurlock, M.E., Roos, M.A., Zuckermann, F.A., Gaskins, H.R., 1999. Weaning anorexia may contribute to local inflammation in the piglet small intestine. J. Nutr. 129, 613-619.

McDonald, D.E., Pethick, D.W., Pluske, J.R., Hampson, D.J., 1999. Adverse effects of soluble non-starch polysaccharide (guar gum) on piglet growth and experimental colibacillosis immediately after weaning. Res. Vet. Sci. 67, 245-250.

McDonald, D.E., Pethick, D.W., Mullan, B.P., Hampson, D.J., 2001. Increasing the viscosity of the intestinal contents alters small intestinal structure and intestinal growth, and stimulates proliferation of enterotoxigenic *Escherichia coli* in newly weaned pigs. Br. J. Nutr. 86, 487-498.

Meijerink, E., Fries, R., Vögeli, P., Masabanda, J., Wigger, G., Stricker, C., Neuenschwander, S., Bertschinger, H.U., Stranzinger, G., 1997. Two alpha(1,2) fucosyltransferase genes on porcine chromosome 6q11 are closely linked to the blood group inhibitor (S) and *Escherichia coli* F18 receptor (ECF18R) loci. Mamm. Genome 8, 736-741.

Michel, P., Rérat, A., 1998. Effect of adding sugar beet fibre and wheat bran to starch diet on the absorption kinetics of glucose, amino-nitrogen and volatile fatty acids in the pig. Reprod. Nutr. Develop. 38, 49-68.

Mikkelsen, L.L., Naughton, P.J., Hedemann, M.S., Jensen, B.B., 2004. Effects of physical properties of feed on microbial ecology and survival of *Salmonella* enterica serovar Typhimurium in the pig gastrointestinal tract. Appl. Environ. Microbiol. 70, 3485-3492.

Molist, F., Gómez de Segura, A., Gasa, J., Hermes, R.G., Pérez, J.F., 2008. Effects of wheat bran level and particle size on the intestinal microbiota composition and activity of early weaned piglets. J. Anim. Sci. 86, S2.

Molist, F., Gómez de Segura, A., Gasa, J., Hermes, R.G., Manzanilla, E.G., Anguita, M., Pérez, J.F., 2009a. Effects of the insoluble and soluble dietary fibre on the physicochemical properties of digesta and the microbial activity in early weaned piglets. Anim. Feed Sci. Technol. 149, 346-53.

Molist, F., Gómez de Segura, A., Perez, J.F., Bhandari, S.K., Krause, D.O., Nyachoti, C.M., 2009b. Effect of wheat bran on the health and performance of weaned pigs challenged with *Escherichia coli* $K88^+$. Livest. Sci. (In the Press).

Molist, F., Ywazaki, M., Gómez de Segura, A., Hermes, R.G., Gasa, J., Pérez, J.F., 2010a. Administration of loperamide and addition of wheat bran to the diets of weaner pigs decrease the incidence of diarrhoea and enhance their gut maturation. Br. J. Nutr. 92, 419-427.

Molist, F., Hermes, R.G., Gómez de Segura, A., Martín-Orúe, S.M., Gasa, J., Manzanilla, E.G., Pérez, J.F., 2010b. The interaction between wheat bran and therapeutic doses of zinc oxide may reduce their effects on the intestinal microbiota of early weaning piglets. Br. J. Nutr. (Under Revision).

Montagne, L., Pluske, J.R., Hampson, D.J., 2003. A review of interactions between dietary fibre and the intestinal mucosa, and their consequences on digestive health in young and non-ruminant animals. Anim. Feed Sci. Technol. 108, 95-117.

Montagne, L., Cavaney, F.S., Hampson, D.J., Lallès, J.P., Pluske, J.R., 2004. Effect of diet composition on postweaning colibacillosis in piglets. J. Anim. Sci. 82, 2364-2374.

Montagne, L., Boudry, G., Favier, C., Le Huerou-Luron, I., Lallès, J.P., Seve, B., 2007. Main intestinal markers associated with the changes in gut architecture and function in piglets after weaning. Br. J. Nutr. 97, 45-57.

Moon, H.W., Kohler, E.M., Scheider, R.A., Whipp, S.C., 1980. Prevalence of pilus antigens, enterotoxin types and enteropathogenicity among K88-negative enterotoxigenic *Escherichia coli* from neonatal pigs. Infect. Immun. 27, 222-230.

Morales, J., Pérez, J.F., Baucells, M.D., Mourot, J., Gasa, J., 2002. Comparative digestibility and lipogenic activity in Landrace and Iberian finishing pigs fed ad libitum corn- and corn-sorghum-acorn-based diets. Livest. Prod. Sci. 77, 195-205.

Morrow, A.L., Ruiz-Palacios, G.M., Jiang, X., Newburg, D.S., 2005. Human-milk glycans that inhibit pathogen binding protect breast-feeding infants against infectious diarrhea. J. Nutr. 135, 1304-1307.

Mouricout, M., Petit, J.M., Carias, J.R., Julien, R., 1990. Glycoprotein glycans that inhibit adhesion of *Escherichia coli* mediated by K99 fimbriae: treatment of experimental colibacillosis. Infect. Immun. 58, 98-106.

Noblet, J., Shi, X.S., Dubois, S., 1994. Effect of body weight on net energy value of feeds for growing pigs. J. Anim. Sci. 72, 648-657.

Noblet, J., Bach Knudsen, K.E., 1997. Comparative digestibility of wheat, maize and sugar beet pulp non-starch polysaccharides in adult sows and growing pigs, in: Digestive Physiology in Pigs, Publ. 88. Eur. Assoc. Anim. Prod., Saint Malo, France, pp. 571.

Odle, J., Zijlstra, R.T., Donovan, S.M., 1996. Intestinal effects of milkborne growth factors in neonates of agricultural importance. J. Anim. Sci. 74, 2509-2522.

Ofek, I., Goldhar, J., Sharon, N., 1996. Anti-*Escherichia coli* adhesin activity of cranberry and blueberry juices. Adv. Exp. Med. Biol. 408, 179-183.

O'Connell, J.M., Callan, J.J., O'Doherty, J.V., 2005. The interaction between cereal type and lactose level on piglet performance and diet digestibility post weaning. Anim. Sci. 81, 265-269.

Oufir, L.E., Barry, J.L., Flourie, B., Cherbut, C., Cloarec, D., Bornet, F., Galmiche, J.P., 2000. Relationships between transit time in man and in vitro fermentation of dietary fiber by fecal bacteria. Eur. J. Clin. Nutr. 54, 603-609.

Partanen, K.H., Mroz, Z., 1999. Organic acids for performance enhancement in pig diets. Nutr. Res. Rev. 12, 117-145.

Petrovic, V., Novotny, J., Hisira, V., Link, R., Leng, L., Kovac, G., 2009. The impact of suckling and post-weaning period. Acta Vet. Brno 78, 365-371.

Piel, C., Montagne, L., Sève, B., Lallès, J.P., 2007. Dietary fibre and indigestible protein increase ileal glycoprotein output without impacting colonic crypt globet cells in weaned piglets. Livest. Sci. 108, 106-108.

Pierce, K.M., Callan, J.J., McCarthy, P., O'Doherty, J.V., 2005. Performance of weanling pigs offered low or high lactose diets supplemented with avilamycin or inulin. Anim. Sci. 80, 313-318.

Pierce, K.M., Callan, J.J., McCarthy, P., O'Doherty, J.V., 2007. The interaction between lactose level and crude protein concentration on piglet post-weaning performance, nitrogen metabolism, selected faecal microbial populations and faecal volatile fatty acid concentrations. Anim. Feed Sci. Technol. 132, 267-282.

Pigman, W., Horton, D., 1972. Structure and stereochemistry of the monosaccharides, in: Pigman W., Horton D. (Eds.), The carbohydrates: chemistry and biochemistry. Academic Press, New York, pp. 1-67.

Plotsky, P.M., Meaney, M.J., 1993. Early, postnatal experience alters hypothalamic corticotrophin-releasing factor mRNA, median eminence CRF content and stress-induced release in adult rats. Mol. Brain Res. 18, 195-200.

Pluske, J.R., Williams, I.H., Aherne, F.X., 1995. Nutrition of the neonatal pig, in: Varley M.A. (Ed.), The Neonatal Pig: Development and Survival. CAB International, Wallingford, Oxon, UK, pp. 187-235.

Pluske, J.R., Williams, I.H., 1996a. Reducing stress in piglets as a means of increasing production after weaning: administration of amperozide or co-mingling of piglets during lactation?. Anim. Sci. 62, 121-130.

Pluske, J. R., Williams, I.H., Aherne, E.X., 1996b. Maintenance of villous height and crypt depth in piglets by providing continuous nutrition after weaning. Anim. Sci. 62, 131-144.

Pluske, J.R., Williams, I.H., Aherne, E.X., 1996c. Villous height and crypt depth in piglets in response to increases in the cow's milk after weaning. Anim. Sci. 62, 145-158.

Pluske, J.R., Hampson, D.J., Williams, I.H., 1997. Factors influencing the structure and function of the small intestine in the weaned pig: a review. Livest. Prod. Sci. 51, 215-236.

Pluske, J.R., Black, B., Pethick, D.W., Mullan, B.P., Hampson, D.J., 2003. Effects of different sources of dietary fibre in diets on performance, digesta characteristics and antibiotic treatment of pigs after weaning. Anim. Feed Sci. Technol. 107, 129-142.

Pond, W.G., Pond, K.R., Ellis, W.C., Matis, J.H., 1986. Markers for estimating digesta flow in pigs and the effects of dietary fiber. J. Anim. Sci. 63, 1140-1149.

Prescott, L.M., Harley, J.P., Klein, D., 1996. Microbiology, third edition. WCB/ McGraw-Hill, Boston, USA.

Ralet, M.C., Faulds, C.B., Williamson, G., Thibault, J.F., 1990. Influence of extrusion-cooking on the physicochemical properties of wheat bran. Carbohydr. Res. 263, 257-269.

Roediger, W.E.W., 1982. Utilisation of nutrients by isolated epithelial cells of the rat colon. Gastroenterol. 83, 424-429.

Russell, J.B., Diez-Gonzalez, F., 1998. The effects of fermentation acids on bacterial growth. Adv. Microbiol. Physiol. 39, 205-234.

Savage, D.C., 1986. Gastrointestinal microflora in mammalian nutrition. Ann. Rev. Nutr. 6, 155-178.

Schiavon, S., Bortolozzo, A., Bailoni, L., Tagliapetra, F., 2004. Effects of sugar beet pulp on growth and health status of weaned piglets. Ital. J. Anim. Sci. 4, 337-351.

Schneeman, B.O., Richter, B.D., Jacobs, L.R., 1982. Response to dietary wheat bran in the exocrine pancreas an intestine of rats. J. Nutr. 112, 283-286.

Schneeman, B.O., 1999. Fiber, inulin and oligofructose: Similarities and differences. J. Nutr. 129, 1424S-1427S.

Selvendran, R.R., 1984. The plant cell wall as a source of dietary fiber: chemistry and structure. Am. J. Clin. Nutr. 39, 320-337.

Sharon, N., Lis, H., 1989. Lectins as cell recognition molecules. Science 246, 227-234.

Simmen, F.A., Whang, K.Y., Simmen, R.C.M., Peterson, G.A., Bishop, M.D., Irvin, K.M., 1990. Lactational variation and relationship to postnatal growth of insulin-like growth factor-1 in mammary secretions from genetically diverse sows. Dom. Anim. Endocrinol. 7, 199-206.

Snoeck, V., Verdonck, F., Cox, E., Goddeeris, B.M., 2004. Inhibition of adhesion of F18+ *Escherichia coli* to piglet intestinal villous enterocytes by monoclonal antibody against blood group H-2 antigen. Vet. Microbiol. 100, 241-246.

Solà-Oriol, D. 2008. Quantitative evaluation of the palatability of feed ingredients in swine. PhD Diss. Universitat Autònoma de Barcelona, Bellaterra, Spain.

Spring, P., Wenk, C., Dawson, K.A., Newman, K.E., 2000. The effects of dietary mannanoligosaccharides on cecal parameters and the concentrations of enteric bacteria in the ceca of salmonella-challenged broiler chicks. Poult. Sci. 79, 205-211.

Stein, H.H., Kil, D.Y., 2006. Reduced use of antibiotic growth promoters in diets fed to weanling pigs: Dietary tools, part 2. Anim. Biotechnol. 17, 217-231.

Stewart, C.S., 1997. Microorganisms in hindgut fermentors, in: Mackie, R.I., White B.A., Isaacson, R.E. (Eds.), Gastrointestinal Microbiology. Chapman and Hall, New York, USA, pp. 142-186.

Su, Y., Yao, W., Perez-Gutierrez, O.N., Smidt, H., Zhu, W.Y., 2008. Changes in abundant of *Lactobacillus spp.* and *Streptococcus suis* in the stomach, jejunum and ileum of piglets after weaning. FEMS Microbiol. Ecol. 66, 546-555.

Swords W.E., Wu, C.C., Champlin, F.R., Buddington, R.K., 1993. Postnatal changes in selected bacterial groups of the pig colonic microflora. Biol. Neonate 63, 191-200.

Thibault, J.F., Ralet, M.C., Axelos, M.A.V., Della Valle, G., 1992. Effects of extrusion cooking on pectin-rich materials. Proc. pectines and pectinases. Wageningen.

Trowell, H., Southgate, D.A., Wolever, T.M., Leeds, A.R., Gassull, M.A., Jenkins, D.J.,1976. Letter: dietary fibre refined. Lancet 1, 1967-1968.

Valencia, Z., Chavez, E.R., 2002. Phytase and acetic acid supplementation in the diet of early weaned piglets: effect on performance and apparent nutrient digestibility. Nutr. Res. 22, 623-632.

Van der Poel, A.F.B., Den Hartog, L.A., Van Stiphout, W.A.A., Bremmers, R., Huisman, J., 1990. Effects of extrusion of maize on ileal and faecal digestibility

of nutrients and performance of young piglets. Anim. Feed Sci. Technol. 29, 309-320.

Van der Waaij, D., 1989. The ecology of the human intestine and its consequences for the over-growth o pathogens such as *Clostridium difficile*. Ann. Rev. Microbiol. 43, 69-87.

Van Leeuwen, P., Versantvoort, C.H.M., 1999. Functional histology of the small intestinal mucosa in livestock production animals, in: Jansmanm, A.J.M, Huisman, J. (Eds.), Nutrition and Gastrointestinal Physiology-Today and Tomorrow. TNO, Wageningen, The Netherlands, pp. 9-21.

Van Nevel, C.J., Dierick, N.A., Decuypere, J.A., De Smet, S.M., 2006. *In vitro* fermentability and physicochemical properties of fibre substrates and their effect on bacteriological and morphological characteristics of the gastrointestinal tract of newly weaned piglets. Arch. Anim. Nutr. 60, 477-500.

Von Engelhardt, W., Rönnau, K., Rechkemmer, G., Sakata, T., 1989. Absorption of short-chain fatty acids and their role in the hindgut of monogastric animals. Anim. Feed Sci. Technol. 23, 43-53.

Wang, J.F., Zhu, Y.H., Li, D.F., Wang, M., Jensen, B.B., 2004. Effect of type and level of dietary fibre and starch on ileal and faecal microbial activity and short-chain fatty acid concentrations in growing pigs. Anim. Sci. 78, 109-117.

Wellock, I.J., Houdijk, J.G.M., Kyriazakis, I., 2007. Effect of dietary non-starch polysacharide solubility and inclusion level on gut health and the risk of post weaning enteric disorders in newly weaned piglets. Livest. Sci. 108, 186-189.

Willemsen, P.T, de Graaf, F.K., 1992. Age and serotype dependent binding of K88 fimbriae to porcine intestinal receptors. Microbiol. Pathog. 12, 367-375.

Williams, B.A., Verstegen, M.W.A., Tamminga, S., 2001. Fermentation in the large intestine of single stomached animals and its relationship to animal health. Nutr. Res. Rev. 14, 207-227.

Wilson, R.A., Francis, D.H., 1986. Fimbriae and enterotoxins associated with *Escherichia coli* serogroups isolated from pigs with colibacillosis. Am. J. Vet. Res. 47, 213-217.

Wolin, M.J., Miller, T.L., 1983. Carbohydrate fermentation, in: Hentges, D.A. (Ed.), Human intestinal flora in health and disease. Academic Press, New York, USA, pp. 147-165.

Worsaae, H., Schmidt, M., 1980. Plasma cortisol and behaviour in early weaned piglets. Acta Vet. Scand. 21, 640-657.

Zimmerman, B., Bauer, E., Mosenthin, R., 2001. Pro- and prebiotics in pig nutrition-potential modulators of gut health?. J. Anim. Feed Sci. 10, 47-56.

Zoetendal, E.G., Akkermans, A.D.L, Akkermans-van Vliet, W.M., Arjan, J.A., De Visser, G.M., De Vos, W.M., 2001. The host genotype affects the bacterial community in the human gastrointestinal tract. Microb. Ecol. Health Dis. 13, 129-134.

CURRICULUM VITAE

PERSONAL INFORMATION

Name	Molist Gasa, Francesc
Address	-
Telephone	-
E-mail (s) and Web Site	francesc.molist@gmail.com
Nationality	Spain
Date of birth	14, 05, 1982

WORK EXPERIENCE

• Dates	01.09.2008 – 31. 08. 2010
• Name of employer	Universitat Autònoma de Barcelona
• Position held	Ph. D student
• Objectives	Development of projects in the Animal Production and Food Science Area
• Dates	01.09.2006 – 31. 08. 2008
• Name of employer	Secretaria de Estado de Universidades e Investigación
• Position held	Ph. D student
• Objectives	Development of projects in the Animal Production and Food Science Area
• Dates	01.09.2005 – 31. 12. 2005
• Name of employer	Universitat Autònoma de Barcelona
• Position held	Research Assistant
• Objectives	Assistant in the project development
• Dates	22.08.05 – 26.08.05
• Name of employer	Explotacions Pecuaries Cove, S.A.
• Position held	Farm attendant
• Objectives	Perform practical work on pig farm
• Dates	20.06.00 – 28. 07. 2000 and 28.08.00 – 15.09.00
• Name of employer	CATAPLASTIC, S.L
• Position held	Employee
• Objectives	Summer work

ACADEMIC DEGREES

• Dates	2010
• Name of organization	Universitat Autònoma de Barcelona
• Title of qualification awarded	European Degree of Doctor of phylosophy (Ph.D) in Veterinary Medicine
• Dates	2007
• Name of organization	Universitat Autònoma de Barcelona
• Title of qualification awarded	Master in Animal and Food Science

• Dates	2005
• Name of organization	Universitat Autònoma de Barcelona
• Title of qualification awarded	Veterinary Medicine Degree

RESEARCH EXPERIENCE

• Dates	2009 – 2010
• Name of organization	Riddet Institute, New Zealand
• Title of project	Effect of kiwi fibre on the intestinal microbiota of cannulated pigs
• Dates	2008 – 2009
• Name of organization	Dpt. of Animal Sciences, Graduate School of Life and Environmental Sciences, Kyoto Prefectural University, Japan
• Title of project	Host-specificity reaction between lactobacilli and white blood cells
• Dates	2007 – 2008
• Name of organization	Dpt. of Animal Sciences, Faculty of Agricultural and Food Sciences, University of Manitoba, Canada
• Title of project	Effect of wheat bran on the health and performance of weaned pigs challenged with *E. coli* K88
• Dates	2006 – 2007
• Name of organization	Dpt. of Biological and Environmental Sciences, Faculty of Biosciences, University of Helsinki, Finland
• Title of project	Inhibition of enterotoxigenic *E.coli* K88ac adhesion to piglet intestinal epithelium by milk components and lactobacilli
• Dates	2006 – 2010
• Name of organization	Universitat Autònoma de Barcelona
• Title of project	Feeding Strategies for enhancing a healthy gut in early weaned pigs (AGL2005-07438-C02-01)

STAYS ABROAD

• Dates	2010 (2 months)
• Name of organization, country	Riddet Institute, New Zealand
• Occupation	Ph.D
• Dates	2009 (3 months)
• Name of organization, country	Dpt. of Animal Sciences, Graduate School of Life and Environmental Sciences, Kyoto Prefectural University, Japan
• Occupation	Ph.D Student
• Dates	2008 (4.5 months)
• Name of organization, country	Dpt. of Animal Sciences, Faculty of Agricultural and Food Sciences, University of Manitoba, Canada
• Occupation	Ph.D Student

• Dates	2007 (6 month)
• Name of organization, country	Dpt. of Biological and Environmental Sciences, Faculty of Biosciences, University of Helsinki, Finland
• Occupation	Ph.D Student

RESEARCH PUBLICATIONS

Molist, F., Hermes, R.G., Gómez de Segura, A., Martín-Orúe, S.M., Gasa, J., Manzanilla, E.G., Pérez, J.F. 2010. The interaction between wheat bran and pharmacological doses of zinc oxide may reduce their effects on the intestinal microbiota of early weaning piglets. British Journal of Nutrition, Under revision.

Molist, F., Gómez de Segura, A., Pérez, J.F., Bhandari, S.K., Krause, D.O., Nyachoti, C.M. 2009. Effect of wheat bran on the health status and performance of weaned pigs challenged with *Escherichia coli* K88+. Livestock Science, pending publication.

Hermes, R.G., Molist, F., Ywazaki, M., Gómez de Segura, A., Gasa, J., Torrallardona, D., Pérez, J.F. 2009. Effects of type of cereal and fibre level on growth and parameters of digestive maturation in young pigs. Livestock Science, pending publication.

Molist, F., Ywazaki, M., Gómez de Segura, A., Hermes, R.G., Gasa, J., Pérez, J.F. 2009. Administration of loperamide and addition of wheat bran to the diets of weaner pigs decrease the incidence of diarrhoea and enhance their gut maturation. British Journal of Nutrition, 103, 879-885.

Hermes, R.G., Molist, F., Ywazaki, M., Nofrarías, M., Gómez de Segura, A., Gasa, J., Pérez, J.F. 2009. Effect of dietary level of protein and fiber on the productive performance and health status of piglets. Journal of Animal Science, 87, 3569-3577.

Molist, F., Gómez de Segura, A., Gasa, J., Hermes, R.G., Manzanilla, E.G., Anguita, M., Pérez, J.F. 2009. Effects of dietary fibre on physicochemical characteristics of digesta, microbial activity and gut maturation in early weaned piglets. Animal Feed Science and Technology, 149, 346-357.

Molist, F., Gómez de Segura, A., Manzanilla, E.G., Gasa, J., Hermes, R.G., Pérez, J.F. 2008. Inclusión de salvado de trigo y pulpa de remolacha en la ración. Albéitar, 117, 50-53.

RESEARCH ABSTRACTS

Guerra, A.A., Molist, F., Hermes, R.G., Gómez de Segura, A., Franco, R., Calvo, M.A., La Ragione, R.M., Woodward, M.J., Pérez, J.F., Martín-Orúe, S. 2010. Effect of lactulose, Lactobacillus plantarum and their combination on the intestinal environment and performance of piglets in the post-weaning period. International Scientific Conference on Prebiotics and Probiotics, Kosice (Slovakia).

Molist, F., Hermes, R.G., Pérez, J.F., Martín-Orúe, S. 2010. A wheat bran extract shows a high attachment to Escherichia coli K88 in-vitro. ADSA/ASA Joint Meeting Congress, Denver (USA).

Hermes, R.G., Molist, F., Pérez, J.F., Gómez de Segura, A., Ywazaki, M., Martín-Orúe, S. 2010. Casein glycomacropeptide (CGMP) in the diet of early weaned piglets reduces the Escherichia coli attachment to the intestinal mucosa and increases lactobacilli numbers in digesta. ADSA/ASA Joint Meeting Congress, Denver (USA).

Molist, F., Hermes, R.G., Martín-Orúe, S.M., Gasa, J., Pérez, J.F. 2009. Estudio del bloqueo de la adhesión bacteriana y de la activación del sistema inmunitario de los animales mediante cepas de Lactobacillus spp. I Reunión Red BAL, Granada (Spain).

Molist, F., , Hermes, R.G., Ywazaki, M., Virkola, R., Korhonen, T., Martín-Orúe, S.M., Pérez, J.F. 2009. Estrategias in-vitro para evaluar la adhesión bacteriana sobre el epitelio intestinal en lechones. II Jornadas Avances Metodológicos en el Estudio de la Microbiología Digestiva, Zaragoza (Spain).

Molist, F., Ywazaki, M., Gómez de Segura, A., Hermes, R.G., Gasa, J., Pérez, J.F. 2009. Efectos de incorporar salvado de trigo y modificar el tránsito digestivo sobre la adaptación del lechón recién destetado. XIII Jornadas sobre Producción Animal (ITEA), Zaragoza (Spain).

Hermes, R.G., Molist, F., Ywazaki, M., Gómez de Segura, A., Gasa, J., Torrallardona, D., Pérez, J.F. 2009. Effects of type of cereal and fibre level on growth and parameters of digestive maturation in young pigs. XI International Symposium on Digestive Physiology of Pigs, Montbrió del Camp (Spain).

Molist, F., Gómez de Segura, A., Pérez, J.F., Bhandari, S.K., Krause, D.O., Nyachoti, C.M. 2009. Effect of wheat bran on the health status and performance of weaned pigs challenged with Escherichia coli K88+. XI International Symposium on Digestive Physiology of Pigs, Montbrió del Camp (Spain).

Molist, F., Virkola, R., Gómez de Segura, A., Pérez, J.F., Korhonen, T. 2008.Inhibition of E. coli k88a attachment to piglet ileum epithelium by milk components and lactobacilli. Gut Microbiome Symposium, Clermont-Ferrand (France).

Hermes, R.G., Molist, F., Ywazaki, M., Nofrarías, M., Gómez de Segura, A., Gasa, J., Pérez, J.F. 2008. The effect of dietary levels of protein and fibre on the productive performance and health status of piglets. ADSA/ASA Joint Meeting Congress, Indianapolis (USA).

Molist, F., Gómez de Segura, A., Gasa, J., Hermes, R.G., Pérez, J.F. 2008. Effects of wheat bran level and particle size on the intestinal microbiota composition and activity of early weaned pigs. ADSA/ASA Joint Meeting Congress, Indianapolis (USA).

Molist, F., Gómez de Segura, A., Gasa, J., Hermes, R.G., Pérez, J.F. 2008. Effect of wheat bran and zinc oxide on the microbiota of weanling pigs. ADSA/ASA Joint Meeting Congress, Indianapolis (USA).

Molist, F., Castillo, M., Gómez de Segura, A., Pérez, J.F., Gasa, J., Martín-Orúe, S.M. 2007. Aplicación de la PCR a tiempo real para la cuantificación de grupos microbianos en el tracto digestivo del cerdo. I Jornadas Avances Metodológicos en el Estudio de la Microbiología Digestiva, Zaragoza (Spain).

Molist, F., Gómez de Segura, A., Manzanilla, E.G., Gasa, J., Hermes, R.G., Pérez, J.F. 2007. Efecto del salvado de trigo y de la pulpa de remolacha en la ración sobre la microbiota intestinal y la maduración digestiva en lechones recién destetados. Tierras de Castilla y León: Ganadería, 140, 88-90. XII Jornadas sobre Producción Animal (ITEA), Zaragoza (Spain).

Molist, F., Gómez de Segura, A., Manzanilla, E.G., Gasa, J., Hermes, R.G., Pérez, J.F. 2007. Post-weaning development of the microbiota composition and activity in piglets fed diets with wheat bran, wheat middlings or sugar beet pulp. Journal of Animal Science, 85, 81. ADSA/ASA Joint Meeting Congress, Sant Antonio (USA).

PERSONAL SKILLS AND COMPETENCES

MOTHER TONGUE	CATALAN
OTHER LANGUAGES	
	SPANISH
• Reading skills	EXCELLENT
• Writing skills	EXCELLENT
• Verbal skills	EXCELLENT
	ENGLISH
• Reading skills	EXCELLENT
• Writing skills	EXCELLENT
• Verbal skills	EXCELLENT
TECHNICAL SKILLS AND COMPETENCES	Windows MS Office and related programs user. Experience in statistic analysis using SAS system. Experience in molecular biology, gas chromatography, citometry, and general chemical analysis
FELLOWSHIPS	Foreign Fellowship - Spanish Government, 2010 Foreign Fellowship - Spanish Government, 2009 Foreign Fellowship - Spanish Government, 2008 Foreign Fellowship - Spanish Government, 2007 Pre-doctoral Fellowship - Spanish Government, 2006 University Department Collaboration Fellowship - Spanish Government, 2005
DRIVING LICENCE(S)	I am a holder of a Spanish driving license. Category B vehicle