Fermentation of liquid diets for pigs

Promotor: **Prof. Dr. Ir. M.W.A. Verstegen**
Hoogleraar in de Veevoeding, in het bijzonder de voeding van eenmagigen, Wageningen Universiteit

Co-promotoren: **Dr. Ir. L.A. den Hartog**
Directeur R&D-Agri Nutreco

Dr. Ir. J.W. Schrama
Universitair Docent, Wageningen Universiteit, Departement Dierwetenschappen, Leerstoelgroep Visteelt en Visserij

Fermentation of liquid diets for pigs

Ronald Scholten

Proefschrift
ter verkrijging van de graad van doctor
op gezag van de rector magnificus
van Wageningen Universiteit,
Prof. Dr. Ir. L. Speelman,
in het openbaar te verdedigen
op dinsdag 27 november 2001
des namiddags te vier uur in de Aula

Scholten, R.H.J., 2001, Fermentation of liquid diets for pigs.
Ph.D. Thesis, Wageningen Institute of Animal Science, Department of Animal Nutrition, Wageningen University, P.O. Box 338, 6700 AH Wageningen, The Netherlands

ISBN 90-5808-524-4

The research described in this thesis was conducted at the Research Institute for Animal Husbandry, P.O. Box 2176, 8203 AD Lelystad, The Netherlands.

NN08201, 309

Stellingen

1. Opname van prégefermenteerde grondstoffen in het biggenvoer heeft, net als de toevoeging antibiotica aan biggenvoeders, een positief effect op de groei en voederconversie van gespeende biggen. Prégefermenteerde grondstoffen zijn een alternatief voor het gebruik van antibiotica in het biggenvoer.
Dit proefschrift.

2. Een gespeende big van circa 5 weken oud consumeert per kg metabool gewicht evenveel alcohol als een mens van 85 kg die dagelijks twee glazen bier drinkt.
Dit proefschrift.

3. Het berekende energieverlies tengevolge van de fermentatie van een koolhydraatrijke grondstof wordt gecompenseerd door een geringere onderhoudsbehoefte van de gespeende big.
Dit proefschrift.

4. Hoewel het gezegde luidt dat "wat in het vat zit, verzuurt niet" blijkt dat koolhydraten die in het vat zitten snel verzuren.
Dit proefschrift.

5. Het Nederlandse varken hergebruikt op jaarbasis circa 3 miljard kg vochtrijke bijproducten uit de humane levensmiddelenindustrie. De varkenshouderij is daarmee een onmisbare schakel in de natuurlijke kringloop van voedingsstoffen.

6. De samenvoeging van alle op de veehouderij georiënteerde onderzoeksinstituten tot één onderneming lijkt tegenstrijdig met de wetten van de mededingingsautoriteiten.

7. Na dramatische gebeurtenissen als de vuurwerkramp in Enschede (13 mei 2000) of de terroristische aanslag op het WTC (11 september 2001) moeten alle sportwedstrijden voor minimaal één week worden afgelast.

Stellingen behorende bij het proefschrift:
Fermentation of liquid diets for pigs.
Ronald Scholten, 27 November 2001

Voor Pa en Hermien

For Letti

In Memoriam:

Ma († 17-5-1987)

Peter Scholten († 13-1-1998)

Voorwoord

Dit proefschrift is het resultaat van ruim 4,5 jaar noeste arbeid. Een fulltime baan werd gecombineerd met een promotieonderzoek. Zonder de hulp van vele mensen was deze combinatie absoluut onmogelijk geweest. Voor hen een persoonlijk woord van dank.

Allereerst de begeleiders van het onderzoek: prof. dr. ir. M.W.A. Verstegen, dr. ir. L.A. den Hartog, dr. ir. J.W. Schrama, ir. C.M.C. van der Peet-Schwering en dr. P.C. Vesseur. Martin, jouw enthousiasme, goede humeur en geniale ideeën zijn bewonderenswaardig. Ondanks je drukke werkzaamheden, had je altijd een luisterend oor voor zaken aangaande mijn promotie en ontwikkelingen in mijn privé leven. Ik ben je zéér erkentelijk voor dit alles, en hoop je nog regelmatig te ontmoeten. Leo, als voormalig directeur van het Praktijkonderzoek Varkenshouderij (PV) heb je me de mogelijkheid geboden om een promotieonderzoek uit te voeren. Het onderwerp "fermentatie van brijvoer voor varkens" was uniek en de resultaten van de experimenten zijn zodanig interessant dat vervolgonderzoek inmiddels gestart is. Leo, jouw helikopterview was uniek en zorgde voor voortgang in de besprekingen en het wegschrijven van de artikelen. Bedankt. Johan en Carola, jullie positief kritische inbreng bij de opzet van de experimenten en de correctie van de artikelen waren zeer waardevol. Mijn eerste concepten waren veelal praktisch geschreven; jullie zorgden voor het 'opkrikken' van het wetenschappelijk niveau. Jullie statistisch inzicht is erg goed. Bij voorkeur laat ik me het juiste statistisch model aanleveren, terwijl ik me liever op de interpretatie van de resultaten richt. De combinatie was dus uitermate efficiënt en plezierig. Peter, door je nieuwe functie kon je de afronding van het geheel niet meemaken. Tijdens mijn periode bij het PV had jij als afdelingshoofd en programmaleider een belangrijke rol in dit promotieonderzoek. Bedankt voor je positieve inbreng.

Eind 1998 werd ik door Beuker Vochtrijke Diervoeders B.V. benaderd om bij hen de functie "hoofd diervoeding en kwaliteitszorg" te vervullen. Kees van Gorp, Johan van Engen en Piet Brokking boden me een dynamische job bij een internationaal opererend en groeiend bedrijf. Tevens kreeg ik de mogelijkheden om mijn promotieonderzoek af te ronden. Ik besefte dat dit een buitenkans was. Hoewel het qua werkdruk niet altijd eenvoudig was, kan ik nu met tevredenheid vaststellen dat het een prima keuze was. In de plezierige en hardwerkende 'no-nonsense' Beuker-familie was het gemakkelijk om vol gas te blijven geven. Alle mensen bij Beuker: hartelijk bedankt!

In de jaren dat ik bij het PV werkte, is een goede kameraadschap met Martin Rijnen en Erik Bruininx ontstaan. Martin, de samenwerking begon toen je participeerde in de 'fermentatieproef'. We hebben in die proef enorm veel werk verzet (ook 's nachts). Mede daardoor was het een perfect project. Helaas kampten we beiden in die periode ook met onbegrijpelijke sterfgevallen in de directe omgeving. Beiden hebben we ons er door heen geslagen. Mede door die periode weten we wat we aan elkaar hebben. Ik wil je ook danken voor de grote inzet die je bij de correctie van mijn artikelen hebt geleverd. Het doet me deugt dat ik je binnenkort als collega bij Beuker Vochtrijke Diervoeders mag verwelkomen. Ik wens je veel succes, zowel privé, als met de afronding van je promotieonderzoek als wel met je nieuwe baan.

Erik, als kamergenoot, moppentapper en collega-visser was het prettig vertoeven met jou. Ons motto luidt "een dag niet gelachen is een dag niet geleefd". Natuurlijk hielden we ons ook met serieuze zaken bezig. Zo beoordeelden we het vrouwelijk schoon, aten regelmatig een pizzaatje JAFFA en hebben we onze hypothese "de schoenmaat van vrouwen" na herhaaldelijke waarnemingen moeten verwerpen. Bedankt voor de leuke tijd, je hulp bij de diverse proeven en de correcties van de artikelen. Ik wens je veel succes met je promotieonderzoek en verdere loopbaan. Thanks, my humble server.

Het personeel van het Varkensproefbedrijf te Sterksel verdient een pluim. Koos Broekman, je was voor mij méér dan de initiator van het onderzoek met vochtrijke diervoeders. Ik heb veel van je geleerd en je toonde ook interesse voor persoonlijke zaken. Ook Anita Hoofs, Mart Smolders, Daniëlle van de Loo en Anton van Zeeland wil ik danken voor de goede samenwerking. De 'jongens' van de proefbedrijven te Sterksel, Rosmalen en Raalte ben ik dank verschuldigd voor de uitvoering van de diverse voedingsproeven.

Binnen het PV waren Jan Huiskes en Nico Verdoes de 'sleutelfiguren'. Jan, jij was mijn eerste chef op het PV en het project "mogelijkheden tot het mesten van vleesbeertjes" verliep prima. Vele slachterijen en vleesverwerkende bedrijven heb ik bezocht, wat erg interessant en leerzaam was. Door jouw I&R project in Hongarije hebben we ook nu nog af en toe contact. Bedankt voor alles en ik wens je het allerbeste. Nico, als programmaleider "bijproducten en granen" had je een belangrijke bijdrage aan mijn loopbaan. In korte tijd hadden we een groot aantal projecten gericht op brijvoer, bijproducten en granen. Jouw nuchtere en efficiënte wijze van werken, was voor mij erg plezierig en een punt van herkenning. Bedankt en succes.

Met enige trots kijk ik terug op de biggenproef waarin gedurende vijf maanden, zeven dagen per week, tweemaal per dag vier soorten brijvoer handmatig werden aangemaakt en verstrekt. De hulp van Gisabeth Binnendijk en Hans Diepstraten was daarbij van onschatbare waarde.

Gisabeth, je werklust, collegialiteit en nauwkeurigheid van werken zijn bewonderenswaardig. Hans, je praktische ervaring en werklust bleken goed van pas te komen. Het was een groot genoegen met jullie samen te werken. Succes.

Op het goed geoutilleerde laboratorium van Huug Boer zijn enorme aantallen analyses uitgevoerd. Huug, je had een treffende uitspraak over de hoeveelheid analyses: "dat is een puist werk". Bedankt voor de mogelijkheden die je geboden hebt. Het was klasse! Ook Truus Post, Marjan van 't End, Jane Martine Muijlaart, Meike Booij en Dick Bongers wil ik danken voor de arbeid die zij hebben verricht. Piet van Leeuwen en Tamme Zandstra wil ik danken voor het dissecteren van de biggen. De dagen waren lang, maar mede door jullie erg gezellig. Regelmatig heb ik 'blauw' gelegen van het lachen.

De Leerstoelgroep Microbiologie ben ik dankbaar voor de perfecte mogelijkheden die ze hebben geboden om de vele microbiologische tellingen uit te voeren. Caroline Plugge en Fons Stams zorgden voor de begeleiding. Melike Balk, thanks for carrying out the huge numbers of microbial counts.

The collaboration with Prof. Peter Brooks (University of Plymouth, United Kingdom) and Colm Moran was very interesting and exciting for me. Peter, you are one of the main researchers in the area of fermented diets. I learned a lot from you and hopefully we stay in contact. Colm, you did an excellent job during your stay in Rosmalen. Thanks a lot and I hope your carrier at Alltech will be successful. We stay in touch.

In 1998 en 1999 heb ik tegenslagen in de privé-sfeer moeten verwerken. Mijn speciale dank gaat uit naar Saskia en Benno Schildkamp, twee uitzonderlijke mensen. Jullie waren er voor me en jullie steun was geweldig. Ik heb veel van jullie geleerd. Benno, je bent als een broer voor me. Als 'oudste' broer is het dan ook logisch dat jij reeds vader bent. Zodra Rick wat ouder is, heeft hij een vakantieadres in Hongarije. Jullie zijn altijd van harte welkom! Ik wens jullie veel succes met de bouw van jullie huis op jullie eigen droomplekje. Geniet er van.

Tijdens mijn studietijd in Wageningen, en ook de jaren erna, heb ik een prima tijd in Renkum gehad. Mevrouw van der Veen, ik ben u dankbaar voor de uitstekende zorg. Nog steeds kom ik met plezier bij u een lekker bakje koffie drinken of een preischotel eten. Bedankt voor alles.

Mijn ouders hebben me altijd gestimuleerd het beste uit mezelf te halen. Pa, ik herinner me dat ik in de brugklas van de HAVO van 'deskundigen' het advies kreeg om een opleiding aan de Middelbare Agrarische School te volgen. Volgens hen was dat het hoogst haalbare. Dat leek me een geniaal idee, want dan hoefde ik minder huiswerk te maken en kon ik nog meer voetballen... Achteraf ben ik blij dat je dit tegenhield. Hermien, voor jou heb ik grote bewondering en diep respect. Je bent een liefdevolle moeder voor me. Pa en Hermien, jullie hebben enorm veel bereikt door hard te werken en met beide voeten op de grond te blijven staan. Die instelling en mentaliteit blijf ik als voorbeeld gebruiken. Ik hou van jullie en ben enorm trots op zulke ouders. Bedankt voor alle steun die ik ten allen tijde van jullie heb mogen ontvangen. Aan jullie, en aan Letti, draag ik dit proefschrift op.

Letti, you gave me back my life and my energy. Your power, love and support are the main reasons that my Ph.D. dissertation is finished yet. Köszönöm szépen édesem. In 1999 I made an important decision in my personal life. It was not an easy decision, but the best and only right one. We are now more than two years together, we engaged and we live together in Hungary, Dánszentmiklós. I want to thank Julika, Ferenc, Feci and Lajos for accepting me and helping me to feel at home in Hungary. I realized that Hungarians are friendly people who still respect the meaning of life and are proud to be Hungarian. Letti, you successfully finished your study at the Agricultural University in Kaposvár. It is now time to realize our dreams and build up a nice future, in private as well as in business. Our new pig farm NOREL KFT is one part of that future. Nagyon szeretlettikém. For you, and my parents, I offer this dissertation.

Ronald

Buurse, The Netherlands
Dánszentmiklós, Hungary

November 2001

Contents

GENERAL INTRODUCTION

General Introduction

In the last 30 years large changes have occurred in pig husbandry and pig nutrition. Until the early 80's it was usual in the Netherlands that pigs were fed manually twice per day using long troughs. Directly after feeding, the feed for the next feeding time was put in the trough already. Water was sprinkled on top of the feed and as a consequence feed could soak (and ferment) several hours till the next feeding time. With the increasing size of the pig farms, manual feeding was replaced by automatic feeding systems. Most pigs were fed on dry diets, but during last 15 years a clear tendency towards liquid feeding systems was observed. In the early 80's the use of liquid co-products from the human food industry was introduced into pig nutrition. The amount of liquid co-products available for use in pig nutrition increased rapidly. In line with that, the number of pigs fed with liquid feed systems increased. In the Netherlands, nowadays about 22.5% of all slaughter pigs and 11% of all sows is fed a liquid diet with one or more liquid co-products (Van Gorp, personal communication). About 75% of the liquid co-products used in Dutch pig nutrition can be classified as carbohydrate-rich and have a high potential for fermentation during storage (Sc. n et al., 1998).

Fermentation is a process whereby starch and sugar i ticular are transformed by microbes into the fermentation products like lactic acid, volatile . y acids (VFA), ethanol and CO_2 (Prescott et al., 1996). Depending on the type of microbes (bacteria, yeasts, moulds) the amount and proportion of the different fermentation products differ (Prescott et al., 1996). Fermentation is regarded as one of the oldest methods of food processing aiming a prolongation of shelf-life and improved palatability. Bread, beer, wine and cheese are well-known examples of fermented human foods, which originate from long before Christ (Anonymous). Although modern food technology has contributed to the present-day high standard of quality and hygiene of fermented foods, the principles of these processes have hardly changed. A variety of fermented foods are very popular with the consumer because of their attractive flavour and their nutritional value.

FERMENTED FEEDSTUFFS IN ANIMAL NUTRITION

Besides the use of fermentation in human nutrition, also in animal nutrition fermented feedstuffs are well-known, e.g. silages of grass, maize and sugar beet pulp. Processing of food for human use yields large quantities of liquid co-products. These products are

increasingly used in pig nutrition (Scholten et al., 1998). It is only some years ago that co-products from the food industry were either discharged into sewers, used as fertilizers on the land, dumped at waste sites, burnt into the atmosphere or dried to 88% dry matter and processed into compound feed (Scholten and Verdoes, 1997). In most countries this still happens. Because of strict environmental policy, however, these methods become either impossible or not attractive, due to restrictions by law and high disposal levies. Alternative outlets for co-products have to be found. Co-products from the food industry generally contain nutrients (easily degradable carbohydrates, fat, protein) that have a high feeding value for pigs. The increasing number of pig farms with automated liquid feed installations and the possibility to reduce feed costs allowed a new market for liquid co-products: the pig industry (Table 1).

Table 1: Amount of liquid co-products (x 1,000,000 kg) used in Dutch pig nutrition (OPNV, 2000)

Industry	1993	1996	1999
Wheat processing	650	885	963
Potato processing	350	525	684
Milk	300	300	500
Brewery	80	100	170
Sugar	25	50	21
Other	250	480	462
Total	**1,655**	**2,340**	**2,800**

Detailed information about the amount of liquid co-products per country is scarce. Despite, it can be estimated that the total amount of liquid co-products used in European pig nutrition is huge. Per year in Denmark 1,800,000 tons (C.F. Hansen, personal communication), in The Netherlands 2,800,000 tons (Table 1), in Northern France about 500,000 tons (Moreau et al., 1992) and Switzerland 1,300,000 tons (Chaubert, 1995) is used in livestock, mainly pigs. Several factors contribute to the expectation that in Europe the use of liquid co-products in animal nutrition will increase further the coming decades. First, the amount of co-products originating from the human food industry which is available for pig nutrition is increasing. This is due to the increase in number of people and the increase in the welfare levels in most

countries. Second, there is increasing pressure to reduce the use of natural energy sources (e.g. gas) and to protect the environment by recycling of minerals.

Although there is a wide range of products available, liquid wheat starch, potato steam peels and whey are the most used liquid co-products (Scholten et al., 1999). The majority of liquid co-products contain high levels of carbohydrates, mainly starch and sugar. In feeding tables (e.g., CVB, 2001) nutrient compositions and nutrient digestibility coefficients from several liquid co-products are published. However, during storage of carbohydrate-rich liquid co-products, fermentation occurs and the characteristics of the co-products changed (Smits, 1998, Scholten et al., 1998). There is a gap in knowledge about the magnitude of the fermentation process and the consequences for the chemical and physical characteristics of the fermented products. The fermentation process might influence the digestibility of the nutrients. Consequently, growth performance (daily gain, feed intake, feed to gain ratio) and health of pigs fed fermented diets, might change. Knowledge on the chemical composition is needed to determine feeding value and to optimise the inclusion of fermented liquid feedstuffs for performance and pig health. Therefore, this research has been focused on the fermentation kinetics of liquid feedstuffs, commonly used in current pig nutrition, and the effect of feeding fermented diets on growth performance, pig health and gastrointestinal characteristics of pigs.

DEFINITION OF THE PROBLEM

Fermentation is a complex process that involves many factors, e.g. time, temperature, amount of type of microbial population, amount and type of substrate available. In human nutrition this process is widely used and well studied. In pig nutrition, however, the process of fermentation of diets is not well studied. As described before, the chemical composition, and the changes in chemical composition, are not well known. The interest of researchers in the use of fermented pig diets increased during the last decade. In general, two possible methods of fermented pig feeds are examined. First, the fermentation of carbohydrate rich liquid co-products is studied. Second, the fermentation of complete compound diets is studied. The fermentation process has consequences for various areas of scientific research, like chemistry, microbiology and physiology. So far, however, studies were mainly focused on growth performance (daily gain, feed intake, feed to gain ratio) of pigs (Russell et al., 1996; Mikkelsen and Jensen, 1997, 1998). Up till now, not much attention has been paid to the fermentation kinetics of liquid pig feeds, commonly used in pig nutrition. Fermentation is

a dynamic process resulting in change of chemical composition as well as physical properties. During fermentation carbohydrates are transformed into lactic acid, volatile fatty acids, ethanol and CO_2 (Prescott et al., 1996). Therefore, storage time may be an important determinant for the magnitude of changes in chemical and physical composition of liquid pig diets. These changes can affect the nutritional value of these pig diets. Information about changes in nutrients, pH, organic acids and ethanol during the fermentation of commonly used pig diets is needed to evaluate changes of feeding value.

Most research has been directed towards the effect of feeding completely fermented liquid compound diets on animals, which had *ad libitum* access to feed. In general, piglets that had *ad libitum* access to fermented diets, showed higher daily feed intake and higher daily gain compared to piglets that had *ad libitum* access to non-fermented liquid diets (reviewed by Jensen and Mikkelsen, 1998). However, no effect on feed to gain ratio was observed (reviewed by Jensen and Mikkelsen, 1998). This means, that despite the fermented diet had lower pH and higher levels of lactic acid and other organic acids, no improvement of the feed to gain ratio was observed. This might be due to protein fermentation, which occurs when complete compound diets, high in protein and amino acids are fermented. Microbes can be easily transform free amino acids into the undesirable components ammonia and toxic amines (Pedersen, 1999, 2001), which are harmful for the gastrointestinal mucosa (Visek, 1972, 1978). During 24 hour fermentation of a liquid compound diet about 25% of the added synthetic lysine disappeared (Pedersen, 1999). This confirms that fermentation of amino acids can occur in liquid compound diets. In contrast to the results reported for fermented compound diets, the addition of individually fermented carbohydrate rich liquid co-products to a liquid pig diet improved daily gain as well as feed to gain ratio (Scholten et al., 1998). Therefore, it is interesting to study in more detail the changes in protein and amino acids during fermentation of individually fermented carbohydrate rich products. It can be suggested that fermentation of individual carbohydrate rich feedstuffs might be a more attractive concept than the fermentation of complete compound diets.

Based on a limited number of studies, pigs fed fermented diets or fermented co-products showed favourable daily gain (Jensen and Mikkelsen, 1998; Scholten et al., 1998), feed to gain ratio (Scholten et al., 1998) and pig health (Scholten et al., 1998) compared to pigs fed non-fermented liquid diets. Fermented diets are characterised by a low pH, high levels of lactic acid and volatile fatty acids, and high amounts of lactic acid bacteria (probiotics). Addition of organic acids to pig diets improve the total tract digestibility of energy and crude

protein (reviewed by Partanen and Mroz, 1999). Furthermore, it is known that short chain fatty acids are beneficial for intestinal epithelial proliferation in rats and pigs (Sakata, 1987; Sakata et al., 1995). This is supported by the fact that villous height in pigs is positively correlated with luminal level of short chain fatty acids (Nousiainen, 1991). Nutrients and level of feed intake after weaning seems to prevent the villous shortening (Makkink, 1993; Van Beers-Schreurs, 1996). In human foods fermentation is used as food conservation but also for improving flavour: lactic acid is used as flavour enhancer (Shelef, 1994). Several trials with the addition of lactic acid to pig diets show that lactic acid increase feed intake (e.g. Roth et al., 1993). Fermented diets, high in lactic and acetic acid, might act beneficial on preventing villous shortening by improved feed intake as well as by the high level of short chain fatty acids. However, there is a gap in the knowledge about the effect of fermented diets on the gastrointestinal characteristics, e.g., level of pH and organic acids, and morphology of the villi in the small intestine.

AIM OF THIS THESIS

The general objective of the research reported in this thesis is to investigate the fermentation of liquid pig diets and the impacts on diet composition, gastrointestinal characteristics and growth performance traits. The specific objectives were:

1. To review literature, on the use, characteristics and effects of fermented diets and feedstuffs on growth performance and pig health and the possible modes of action of fermented feedstuffs;
2. Investigate the fermentation kinetics of complete compound diets during storage under practical conditions;
3. Investigate the fermentation kinetics of individual carbohydrate-rich feedstuffs during storage under practical conditions;
4. Investigate the effect of the addition of a fermented carbohydrate rich feedstuff to liquid weanling diets on a) the growth performance and animal health, and b) the microbial, physiological and morphological aspects of the gastrointestinal tract.

OUTLINE OF THIS THESIS

A review was conducted on fermented liquid co-products and fermented liquid compound diets, and their effects on growth performance and pig health and their possible modes of action. In chapter 1 the review is given.

Based on the conclusions of the review, experiments were conducted to get more detailed information about the fermentation kinetics of different feedstuffs, commonly used in pig nutrition. In the first experiment, five commonly used liquid compound diets and liquid co-products were selected to investigate effects of storage on chemical and physical characteristics of these products. Three carbohydrate rich liquid co-products (liquid wheat starch, potato steam peels and cheese whey) and two liquid compound diets (liquid grower diet and liquid finisher diet) were studied during a six-day storage period. The results of this experiment are described in Chapter 2 and 3. Furthermore, the effect of fermentation on the energy value is examined.

In the results described in Chapter 2 and 3, fermentation characteristics of both liquid compound diets were, for several reasons, less suitable for practical application. Because of experimental application, it was necessary to use a feedstuff which is constant during the duration of the animal trials (about 6 months) and originating from one batch with well-known chemical composition. The use of carbohydrate-rich liquid co-products was less optimal for this experimental application. Therefore, additional research was performed to study the fermentation process of a 'dry' carbohydrate rich feedstuff: wheat. Wheat is the basic for the most used liquid co-product in pig nutrition, liquid wheat starch (Scholten et al., 1999). The fermentation process of hammer milled wheat, mixed with water, was studied. A second objective of this study was to investigate the effect of the addition of a starter culture and the addition of different proportions of pre-fermented wheat (back-slopping). Chapter 4 describes the results obtained from this experiment.

The fermentation of wheat, as described in Chapter 4, showed that within 24 hours a fermented product with a pH below 4 was obtained. To investigate the effect of the addition of fermented wheat to a liquid diet, two experiments were carried out. In a first experiment the effect of fermented liquid wheat on the gastrointestinal microbiology, physiology and morphology in weanling piglets was studied. The results from this experiment are described in Chapter 5. In a second experiment the effect of four levels of fermented wheat on the growth performance and pig health was studied. The results from this experiment are described in Chapter 6. Finally, in the General Discussion, the results of the different experiments, are combined and discussed.

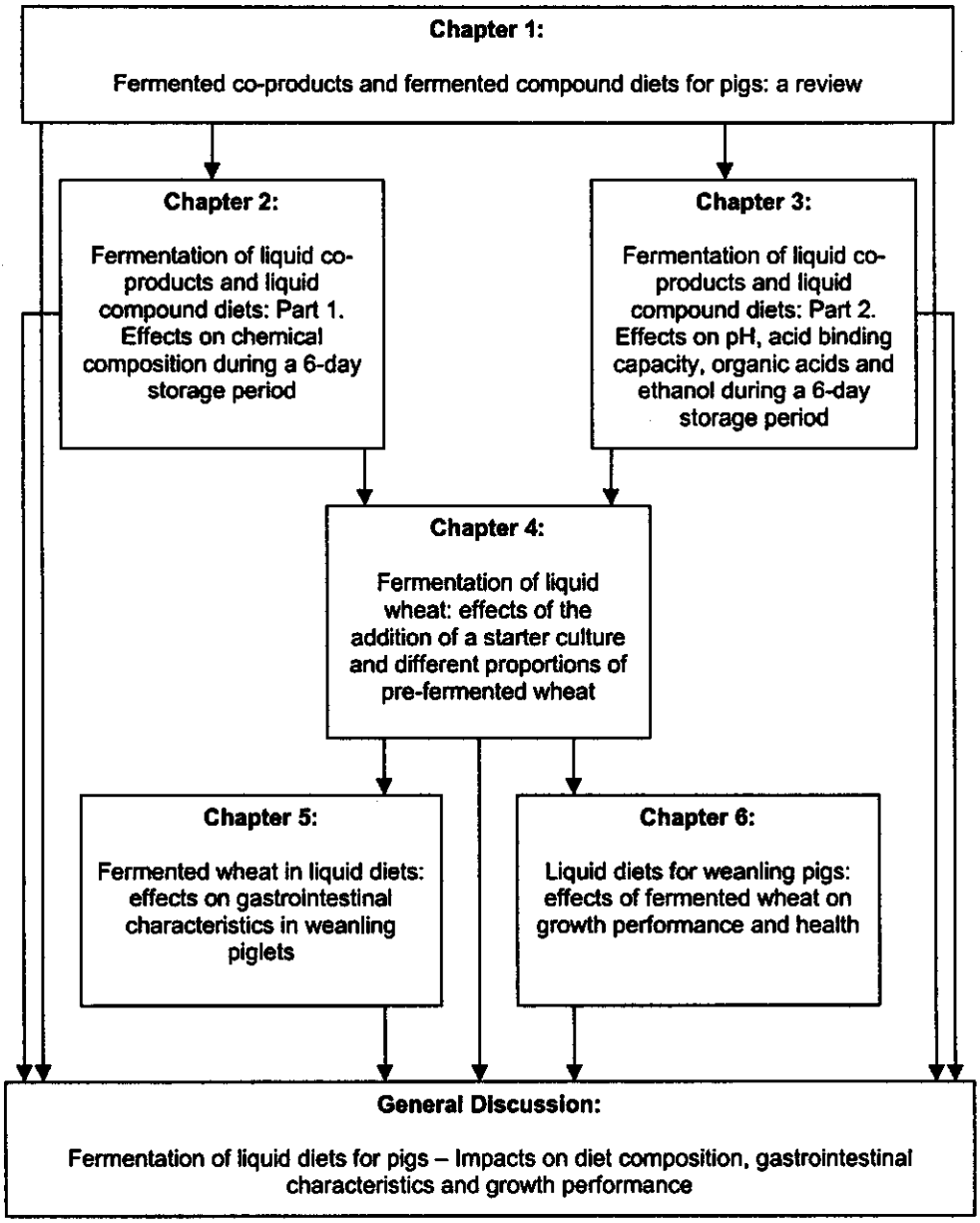

Figure: Schematic representation of this thesis

REFERENCES

Anonymous. Fermentatie van levensmiddelen – Deel 1. Inleiding door M.J.R. Nout, pagina I-1. Dictaat P050-203. Landbouwuniversiteit Wageningen.

Beers-Schreurs, H.G.M. van, 1996. The changes in the function of the large intestine of weaned pigs. Ph.D. thesis, Universiteit Utrecht, Faculteit der Diergeneeskunde, The Netherlands, 149 p.

Chaubert, 1995. Die Nebenprodukte der Lebensmittelherstellung. Agrarforschung 2, 49-52.

CVB, 2001. Centraal Veevoederbureau. Veevoedertabel. Lelystad, the Netherlands.

Jensen, B.B., and L.L. Mikkelsen. 1998. Feeding liquid diets to pigs. In: Garnsworthy, P.C., Wiseman, J. (Eds.), Recent Advances in Animal Nutrition. Nottingham University Press, Loughborough, 107-126.

Makkink, C.A., 1993. Of piglets, dietary proteins and pranceatic proteases. Ph.D. thesis, Department of Animal Nutrition, Agricultural University, Wageningen, The Netherlands, 168 p.

Mikkelsen, L.L., and B.B. Jensen. 1997. Effect of fermented liquid feed (FLF) on growth performance and microbial activity in the gastrointestinal tract of weaned piglets. In: Laplace, J.P., Fevrier, C., Barbeau, A. (Eds.), Digestive Physiology in Pigs. E.A.A.P. publication No. 88, 26-28 May. Saint Malo, France, 639-642.

Mikkelsen, L.L., and B.B. Jensen. 1998. Performance and microbial activity in the gastrointestinal tract of piglets fed fermented liquid feed at weaning. J. Anim. Feed Sci. 7, 211-215.

Moreau, R., Quémeré, P., Carlier, J.C., 1992. Utilisation de sous-produits agro-industriels dans l'alimentation du proc : 1-Pratiques et résultats technico-économiques dans le Nord de la France. Journées Rech. Porcine en France 24, 143-150.

Nousiainen, J., 1991. Comparative observations on selected probiotics and olaquindox used as feed additives for piglets around weaning. 2. Effect on villus height and crypt depth in the jejenum, ileum, caecum and colon. J. Anim. Physiol. Anim. Nutr. 66, 224-230.

OPNV, 2000. Persbericht Overleggroep Producenten Natte Veevoeders.

Partanen, K., Mroz, Z., 1999. Organic acids for performance enhancement in pig diets. Nutr. Res. Rev. 12, 1-30.

Pedersen, A. Ø., 1999. Styret fermentering af vådfoder. Kongres for Svineproducenter, 26-27 Oktober, Kopenhagen, Denmark.

Pedersen, A.Ø., 2001. Fermented liquid feed for weaners. Report no. 510, The National Committee for Pig Production, Danish Bacon and Meat Council, Denmark.

Prescott, L.M., Harley, J.P., Klein, D.A., 1996. Microbiology. WCB Publishers, 935 p.

Roth, F.X., Kirchgessner, M., Eidelsburger, U., 1993. Zur Nutritiven Wirksamkeit von Milchsäure in der Ferkelaufzucht. Agribiol. Res. 46, 229-239.

Russell, P.J., Geary, T.M., Brooks, P.H., Campbell, A., 1996: Performance, water use and effluent output of weaner pigs fed ad libitum with either dry pellets or liquid feed and the role of microbial activity in the liquid feed. J. Sci. Food Agric. 72, 8.

Sakata, T., 1987. Stimulatory effect of short-chain fatty acids on epithelial cell proliferation in the rat intestine: a possible explanation for trophic effects of fermentable fibre, gut microbes and luminal trophic factors. Brit. J. Nutr. 58, 95-103.

Sakata, T., Adachi, M., Hashida, M., Sato, N., Kojima, T., 1995. Effect of n-butyric acid on epithelial cell proliferation of pig colonic mucosa in short-term culture. Dtsch. Tierärtzl. Wschr. 102, 163-164.

Scholten, R., Verdoes, N., 1997. The dutch benefit from a recycling role. Pigs, 13-2, 16-17.

Scholten, R.H.J., Hoofs, A.I.J., Verdoes, N., 1997. By-products related to performance and environmental characteristics at growing/finishing pigs. Proefverslag P1.187. Praktijkonderzoek Varkenshouderij, Rosmalen, The Netherlands (in Dutch with English summary).

Scholten, R.H.J., Peet-Schwering, C.M.C. van der, Hartog, L.A. den, Vesseur, P.C., Verstegen, M.W.A., 1998. Effect of liquid by-products on performance and health of pigs. Paper no. 2.2, presented 49th Annual Meeting of European Association for Animal Production, 24-27 August, Warsaw, Poland.

Shelef, L.A., 1994. Antimicrobial effects of lactates: a review. J. Food Protection 57, 445-450.

Smits, B., 1998. Effect van bewaring en droge stofbepaling op de chemische samenstelling en op de mate van vervluchtiging van vluchtige bestanddelen in vochtrijke enkelvoudige diervoeders (in Dutch). Rapport ID-DLO no. 98.031. Instituut voor Dierhouderij en Diergezondheid. Lelystad.

Visek, W.J., 1972. Effects of urea hydrolysis on cell life-span and metabolism. Fed. Proc. Fed. Am. Soc. Exp. Biol. 31, 1178-1193.

Visek, W.J., 1978. The mode of growth promotion by antibiotics. J. Anim. Sci. 46, 1447-1469.

Chapter 1

FERMENTED CO-PRODUCTS AND FERMENTED COMPOUND DIETS FOR PIGS: A REVIEW

Ronald H.J. Scholten[1,*], Carola M.C. van der Peet-Schwering[1], Martin W.A. Verstegen[2], Leo A. den Hartog[1], Johan W. Schrama[3], Peter C. Vesseur[1]

[1] Research Institute for Animal Husbandry, Lelystad, The Netherlands

[2] Wageningen Institute of Animal Sciences, Animal Nutrition Group, Wageningen, The Netherlands

[3] Wageningen Institute of Animal Sciences, Fish Culture and Fisheries Group, Wageningen, The Netherlands

[*] Present address: Beuker Vochtrijke Diervoeders B.V., Doetinchem, The Netherlands

Published in:

Reprinted from Animal Feed Science and Technology 82 (1999) 1-19

with permission of Elsevier Science

ABSTRACT

This review deals with the properties of fermented diets and their effects on growth performance and gastrointestinal environment of pigs. In addition, some possible modes of action are hypothesized. Starch and sugar rich liquid co-products have a high potential for fermenting during storage. Soaking compound feed with water is another possibility to achieve a fermented diet. These diets are characterized by a pH between 3.5 and 4.5, high levels of lactic acid, and, to a lesser extent, acetic acid and alcohol. Fermented diets seem to improve growth performance of pigs compared with pigs fed non-fermented diets. The exact reasons for this are not yet clear; however some hypotheses are given. Based on a limited number of studies, fermented diets reduce the gastric pH and the number of Coliform bacteria in the gastrointestinal tract compared with non-fermented diets. Furthermore, there are some indications that fermented diets may positively affect pancreatic secretion, villus architecture, digestibility and absorption of dietary nutrients. Fermented diets may reduce the physical activity of pigs. More specific studies on the effect and modes of action of fermented diets are needed to allow firmer conclusions to be drawn.

INTRODUCTION

In European pig nutrition, the recycling of liquid co-products from human food industries has increased over the last decade. Several million tons of liquid co-products, in particular starch and sugar rich co-products, are used annually (Chaubert, 1995; Müller, 1996; Scholten et al., 1998). Feeding liquid co-products to pigs implies that alternative methods of disposal like drying, dumping or burning are no longer necessary. Consequently, the use of fossil energy and the negative effects on the environment are reduced (Scholten and Verdoes, 1997).

Fermentation is a dynamic process whereby starch and sugar in particular can be transformed by microbes into the fermentation products lactic acid, organic acids and alcohol (Prescott et al., 1996). At the moment of delivery to pig farms, starch and sugar rich liquid co-products already have a pH between 3.5 and 4.5 and contain fermentation products (Smits et al., 1996; Scholten et al., 1998; Smits, 1998a). Data from Dutch pig farms using management information systems (Siva-producten B.A., The Netherlands), showed that farms feeding liquid co-products to growing-finishing pigs had a favourable growth performance and mortality rate compared with farms feeding dry compound feed (Scholten and Verdoes, 1997). In addition, pigs fed liquid diets, including fermented co-products, showed an improved daily gain and feed conversion ratio compared with pigs fed liquid diets without these co-products (Scholten et al.,

1998). Moreover, feeding costs decreased by 10 to 17% when liquid co-products were fed compared with dry diets (Siva, 1997; Scholten et al., 1998).

In addition to fermented liquid co-products, fermented diets can also be achieved when dry compound feed is mixed with water and stored for at least 8 hours (Russell et al., 1996; Jensen and Mikkelsen, 1998). Feeding fermented liquid compound diets to weaned piglets improved daily gain and changed the gastrointestinal environment in a more desirable direction compared with non-fermented liquid diets (Russell et al., 1996; Mikkelsen and Jensen, 1997). Fermentation also reduces the growth of undesirable microbes like, Coliforms and *Salmonella spp* (Russell et al., 1996; Jensen and Mikkelsen, 1998). The growth of these potential enteropathogens is reduced at a pH below 4.5. Analyses of pig herds (Dahl, 1997; Van der Wolf et al., 1999) have demonstrated that infections with *Salmonella* are less prevalent in herds fed liquid diets, which, in most cases, contained fermented liquid co-products, compared with herds fed dry diets.

This review starts with a brief summary of the chemical composition and nutrient digestibilities of some starch and sugar rich liquid co-products. It continues with a review of the specific properties of fermented liquid co-products and fermented liquid compound diets and their effects on growth performance and gastrointestinal environment. Wherever possible, modes of action are given or suggested.

CHEMICAL COMPOSITION AND DIGESTIBILITY OF LIQUID CO-PRODUCTS

Definition of liquid co-products

In this review, liquid co-products are defined as "organic co-products originating from the human food industries with a moisture content of at least 600 g/kg". They can be divided into carbohydrate rich, protein rich and fat rich co-products. From the point of view of fermentation, fat and protein are less desirable nutrients. In contrast, starch and sugars can be fermented by microbes, in particular lactic acid bacteria and yeasts, resulting in products such as lactic acid, organic acids, ethanol and CO_2 (Prescott et al., 1996). In this review, starch and sugar rich liquid co-products are defined as "co-products with a total starch and sugar content of at least 400 g/kg dry matter".

Use of liquid co-products in pig nutrition

In European pig nutrition, several million tons of liquid co-products are recycled annually, in particular, the starch and sugar rich co-products liquid wheat starch (LWS), potato steam peel (PSP) and cheese whey (CW). In general, detailed information about the amount and sort of liquid co-products per country is limited. The use of LWS, PSP and CW in Northern France is estimated at about 0.5 x 10^9 kg per year (Moreau et al., 1992). Annually, about 1.3 x 10^9 kg and 1.8 x 10^9 kg of CW are used in pig nutrition in Switzerland (Chaubert, 1995) and Denmark (C.F. Hansen, personal communication) respectively. In 1996 about 2.3 x 10^9 kg of liquid co-products from the human food industries was used in Dutch pig nutrition (Table 1), an increase of more than 40% compared to 1993 (Scholten et al., 1998). About three quarters of the liquid co-products used in the Netherlands can be classified as starch and sugar rich co-products; LWS, PSP and CW are the most frequently used products (Scholten et al., 1998).

Table 1. Amount of liquid co-products (x 1000 kg) from the human food industries delivered to pig farms in the Netherlands (Scholten et al., 1998)

	1993	1996
Wheat starch industry	650 000	885 000
Potato industry	350 000	525 000
Dairy industry	300 000	300 000
Fermentation industry	80 000	120 000
Beer industry	80 000	100 000
Sugar industry	25 000	50 000
Other industry	170 000	360 000
Total amount	**1 655 000**	**2 340 000**

LWS, PSP and CW have a high potential for fermenting during storage (Scholten et al., 1998; Smits, 1998b). Therefore, in this review, attention is focused on these starch and sugar rich liquid co-products. LWS is a co-product resulting from the production of wheat starch and gluten out of wheat flour. LWS is a mixture of starch, protein and fiber (Smits and Jongbloed, 1996). PSP is a co-product resulting from the steam peeling process of potatoes. Potatoes are subjected to pressure and heat of 1.6-1.8 MN/m^2 and 200-210 °C respectively. The cooked peel and a thin layer of cooked starch are removed and these form PSP (Smits and Jongbloed, 1996). CW is a co-product resulting from the production of cheese. The feeding of LWS, PSP

and CW requires specific facilities at farms. These products are delivered to the pig farm as a liquid, with a temperature between 20 °C and 50 °C, stored in tanks over a period of several days or even weeks. During storage LWS and CW are stirred several times per day, whereas, in practice, PSP is not stirred; because of its high viscosity it is not separated (Scholten and Rijnen, 1998). It is suggested that the storage tanks act as fermentation vessels (Scholten et al., 1998; Smits, 1998b).

Chemical composition of LWS, PSP and CW

From the dry matter (DM) content, starch and/or sugar concentrations, LWS, PSP and CW can be regarded as starch and sugar rich liquid co-products (Table 2). The data in Table 2 are derived from samples taken at the moment of delivery to pig farms. The stage of fermentation is unknown, but in general the co-products have already been stored for several hours to days at the factory before they are delivered. On average, DM of LWS, PSP and CW is low. In addition to their low DM content, these products are characterized by a high variability in chemical composition. Starch and sugar content in LWS ranged from 333.3 to 691.6 g/kg DM and 25.6 to 197.3 g/kg DM, respectively, and in PSP from 282.5 to 573.0 g/kg DM and 18.5 to 45.7 g/kg DM, respectively. In CW, the main component is lactose (Table 2).

Nutrient digestibilities in LWS, PSP and CW

Results of digestibility trials conducted with LWS, PSP and CW are summarized in Table 3. Both LWS and PSP exhibited high apparent fecal digestibility coefficients of organic matter and nitrogen-free extract, ranging from 0.89 to 0.98. The crude protein fraction in LWS was highly digestible at 0.90, whereas the digestibility of crude protein in PSP was 0.71. The apparent ileal digestibility of organic matter was 0.86 and 0.71 for LWS and PSP respectively; crude protein ileal digestibility was 0.85 and 0.47, respectively (Smits, 1998b). The ileal digestibility of the essential amino acids ranged from 0.88 to 0.96 and from 0.32 to 0.54 for LWS and PSP, respectively (Smits, 1998b). CW has an apparent faecal digestibility of 0.93, 0.95 and 0.86 for organic matter, nitrogen-free extract and crude protein, respectively (CVB, 1998).

In general, the apparent faecal digestibility of organic matter, nitrogen-free extract and crude protein in LWS, PSP and CW is comparable with, or better than, the apparent fecal digestibility of comparable dry feedstuffs: wheat, potato and whey powder, respectively (Table 3). Therefore, LWS, PSP and CW can be regarded as useful ingredients for pig feeding.

Table 2. Dry matter, starch, sugar and crude protein (g/kg dry matter) content in the fermented liquid co-products liquid wheat starch (LWS), potato steam peel (PSP) and cheese whey (CW) sampled at delivery on pig farms

			Dry matter (g/kg)		Starch		Sugar		Crude Protein	
Liquid co-product	Reference[1]	Batches (n)	average	std[2]	average	std[2]	average	std[2]	average	std[2]
LWS - factory A	a	6	243.0	12.5	427.4	16.0	147.3	27.9	116.2	8.3
LWS - factory B	a	5	213.6	32.3	511.0	94.5	68.4	33.7	111.8	29.5
LWS - factory C	a	5	182.2	12.2	691.6	29.5	25.6	12.3	61.4	10.1
LWS	b	20	250.0	19.0	398.0	48.0	n.a.[3]	n.a.[3]	128.0	20.0
LWS - factory A	c	3	250.0	21.4	333.3	31.5	197.3	14.3	130.0	7.8
LWS - factory B	c	3	221.0	11.0	458.0	34.7	89.0	12.0	100.3	7.4
LWS - factory C	c	3	165.3	10.6	595.0	41.9	55.7	27.5	86.0	2.2
PSP - factory A	a	3	134.3	4.6	444.0	75.7	33.7	15.8	149.7	11.6
PSP - factory B	a	3	130.7	5.6	340.7	79.1	45.7	12.7	148.7	2.9
PSP - factory C	a	2	159.5	1.5	572.0	37.0	18.5	9.5	109.0	3.0
PSP - factory D	a	2	153.0	4.0	490.5	40.5	20.0	4.0	145.0	3.0
PSP - factory E	a	2	153.5	2.5	476.5	12.5	35.5	8.5	145.5	1.5
PSP - factory F	a	2	148.5	2.5	282.5	3.5	32.5	1.5	141.0	0.0
PSP	b	8	153.0	1.0	573.0	84.0	n.a.[3]	n.a.[3]	133.0	8.0
PSP - factory A	c	3	113.7	15.4	317.0	69.3	30.7	10.5	176.0	13.1
PSP - factory B	c	3	135.0	6.7	451.0	24.4	21.7	1.7	146.3	9.5
CW	b	25	60.0	6.0	0.0	0.0	n.a.[3]	n.a.[3]	188.0	30.0
CW	d	unp.[4]	52.0	10.0	0.0	0.0	580.0	unp.[4]	138.0	27.0

[1] a = Smits et al. (1996); b = Scholten et al. (1997); c = Smits (1998); d = CVB (1998).
[2] Standard deviation; [3] Not analyzed; [4] Unpublished.

Table 3. Average apparent fecal and ileal digestibility coefficients of organic matter, crude protein, NFE and 5 essential amino acids of liquid wheat starch (LWS), wheat, mashed potato steam peel (PSP), dried potatoes, cheese whey (CW) and whey powder

	LWS[1]	Wheat[2]	PSP[1]	Potato (dry)[2]	CW[2]	Whey powder[2]
Dry matter (g/kg)	251	861	144	897	52	960
Fecal digestibility						
Organic matter	0.97	0.89	0.89	0.88	0.92	0.94
Crude protein	0.90	0.83	0.71	0.45	0.86	0.80
NFE[3]	0.98	0.92	0.95	0.94	0.94	0.97
Ileal digestibility						
Crude protein	0.85	0.80	0.47	n.p.	n.p.	0.81
Lysine	0.91	0.74	0.54	n.p.	n.p.	0.88
Methionine	0.90	0.85	0.54	n.p.	n.p.	0.86
Cystine	0.91	0.83	0.32	n.p.	n.p.	0.84
Threonine	0.88	0.71	0.41	n.p.	n.p.	0.82
Tryptophan	0.96	0.79	0.37	n.p.	n.p.	0.80

[1] Smits (1998b).
[2] CVB (1998).
[3] Nitrogen-free extract.

SPECIFIC PROPERTIES OF FERMENTED DIETS

Fermented diets can be obtained by storage of starch and sugar rich liquid co-products (Scholten et al., 1998; Smits, 1998b), and by soaking compound feed with water for at least 8 hours (Jensen and Mikkelsen, 1998). Fermented diets have some specific properties compared to non-fermented diets: a) microbial population and b) acidity and level of organic acids, which are described in more detail.

Microbial population

Fermented liquid compound diets have a higher number of lactic acid bacteria (9.6 versus 2.8 log cfu/g diet) and yeasts (6.0 versus 4.3 log cfu/g diet) than non-fermented liquid compound diets (Mikkelsen and Jensen, 1998). In PSP, the amount of lactic acid bacteria and yeasts range from 7.0 to 8.4 log cfu/g and from 3.7 to 6.7 log cfu/g product, respectively (Edwards et al., 1986). Furthermore, high numbers of lactic acid bacteria and yeasts are present in LWS and

CW (Van Gorp, personal communication). It can be assumed that the number of lactic acid bacteria and yeasts will not change very much when dry diets are stored.

Fermentation of carbohydrates by lactic acid bacteria and yeasts results in the production of lactic acid, organic acids, alcohol and CO_2 (Prescott et al., 1996). The microbial population determine both the type and the proportion of fermentation products produced from carbohydrates. Homo-fermentative lactic acid bacteria produce about 0.9 lactic acid, whereas hetero-fermentative species form substantial amounts (0.5) of products other than lactic acid, like acetic acid, ethanol and CO_2. Yeasts will transform carbohydrates into alcohol and CO_2 (Prescott et al., 1996). So far, information about the microbiology of fermented diets and the effect of fermentation on nutritional components of the diets is scarce.

Acidity, organic acids and alcohol

At the moment fermented co-products or fermented compound diets are fed to pigs, they have an average pH between 3.5 and 4.4 (Table 4), whereas the pH of dry diets or non-fermented liquid diets is between 5.5 and 6.1 (Russell et al., 1996; Scholten et al., 1998). On average, lactic acid level of LWS and PSP is high, ranging from 38.0 to 64.4 g/kg DM and from 64.0 to 105.3 g/kg DM, respectively. In LWS, the acetic acid level ranged from 5.6 to 24.3 g/kg DM, whereas in PSP this level ranged from 21.0 to 35.3 g/kg DM. Small amounts of ethanol are also present in the co-products (Table 4). Since fermentation is a dynamic process, the chemical composition of the liquid co-products may alter during storage. The different stages of fermentation at the moment samples are taken, may contribute to the observed variability in chemical composition (Tables 2 and 4). Knowledge on the changes in chemical composition during storage of liquid co-products is scarce. Smits (1998b) observed a decrease in pH and glucose levels concomitant with an increase in lactic acid, acetic acid and alcohol during a three week storage period of LWS and PSP. More specific information about changes in chemical composition of liquid co-products is needed to evaluate changes in feeding value and level of fermentation.

It seems that the proportion of lactic acid to acetic acid is higher in fermented liquid diets than in the fermented liquid co-products (Table 4). This may be the result of a different microbial population between the different products and diets. The absolute level of lactic acid and the proportion of lactic acid to acetic acid may influence diet palatability and, consequently, feed intake. In human food industry lactic acid is used as a flavour enhancer (Shelef, 1994). From a very limited number of experiments it seems that the addition of lactic acid to dry diets increases feed intake in pigs, whereas the effect of acetic acid on feed intake is less clear (Table 5). Moreover, lactic acid or acetic acid supplemented dry compound diets seem to

improve both daily gain and feed conversion ratio, especially when higher levels are added. So far, the effect of the addition of lactic acid or acetic acid to liquid diets on feed intake and growth performance of pigs has not been studied. Also, the combined addition of lactic acid and acetic acid in the proportions commonly present in fermented diets requires investigation. For a good evaluation, the modes of action of these organic acids in liquid diets must be determined also.

Prediction of the energy value of fermented diets

Fermented diets contain organic acids and alcohol (Table 4), that are energy-yielding and which should be taken into account when producing pig diets. However, in most energy formulas this is not the case. Another problem is that some of the fermentation components evaporates when dry matter content is determined at 103°C heating. In the Dutch energy formula, a correction for evaporation, and energy value of lactic acid, volatile fatty acids and alcohol are taken into account (CVB, 1997). The evaporation of lactic acid, volatile fatty acids and alcohol is estimated at 8%, 50% and 100%, respectively. It should be pointed out, however, that more information is need to accurately estimate the rate of evaporation (CVB, 1997).

Table 4. pH and levels of lactic acid, acetic acid and ethanol (g/kg DM) in fermented weaner diets (FWD) and in the fermented liquid co-products, liquid wheat starch (LWS) and potato steam peel (PSP)

Product	Reference[1]	Batches (n)	pH		Lactic acid		Acetic acid		Ethanol	
			average	std[2]	average	std[2]	average	std[2]	average	std[2]
LWS - factory A	a	6	3.6	0.1	62.0	6.9	24.3	15.1	2.7	1.1
LWS - factory B	a	5	3.5	0.1	38.0	7.5	12.8	3.2	7.0	1.9
LWS - factory C	a	5	3.6	0.1	64.4	20.5	5.6	2.9	6.6	5.0
LWS - factory A	b	3	3.5	0.1	61.7	3.1	14.7	6.2	2.7	0.5
LWS - factory B	b	3	3.5	0.2	44.7	13.3	21.3	13.0	10.0	1.4
LWS - factory C	b	3	3.8	0.3	49.7	12.0	13.0	5.7	10.7	1.3
PSP - factory A	a	3	4.2	0.1	64.0	6.7	21.0	7.0	2.7	2.4
PSP - factory B	a	3	4.0	0.2	83.3	22.6	27.3	10.4	5.0	1.6
PSP - factory C	a	8	3.9	0.3	79.1	35.6	23.3	9.8	3.5	3.2
PSP - factory A	b	3	4.0	0.1	105.3	3.4	35.3	2.9	4.7	2.6
PSP - factory B	b	3	3.8	0.2	79.0	20.3	25.0	1.4	1.3	0.5
FWD - 24 hours[3]	c	unp.[4]	4.1	0.0	81.3[5]	12.3[5]	11.5[5]	2.2[5]	unp.	unp.
FWD - 8 hours[3]	d	unp.	4.4	0.2	54.4[5]	4.6[5]	3.6[5]	0.8[5]	unp.	unp.

[1] a: Smits et al. (1996); b: Smits (1998a); c: Mikkelsen and Jensen (1997); d: Mikkelsen and Jensen (1998).
[2] Standard deviation.
[3] Liquid compound diets fermented for 24 hours or 8 hours with the addition of 50% already fermented compound diet.
[4] Unpublished.
[5] Data are recalculated from the original data, which were expressed in mmol/kg liquid diet with 235 g dry matter.

Table 5. Improvements (in % of control) in daily gain, feed intake and feed conversion ratio after the addition of acetic acid (A) and lactic acid (L) to pig diets

Ref[1]	Weight range (kg)	Experimental period (days)	Type acidifier	Dose acidifier (g/kg)	Daily gain	Feed intake	FCR[2]
1	5.5 – 25	46	A	9	-2.1	-1.7	+1.1
				18	+1.2	-0.9	-1.7
				27	+4.0	+0.8	-2.9
2	6.8 – 27.5	42	L	8	+4.7	+6.1	+1.2
				16	+8.1	+6.1	-1.8
				24	+7.3	+5.4	-1.8
3	18 - 45	35	L	16 and 32[3]	+9.7	+4.7	-4.9
				16 and 32[4]	+8.3	+2.8	-5.7

[1] 1: Roth and Kirchgessner (1988); 2: Roth et al. (1993); 3: Jongbloed and Jongbloed (1996).
[2] "-" means that feed conversion ratio is better, whereas "+" means that feed conversion ratio is worse.
[3] No significant difference in doses of supplemented lactic acid was observed. Diets without extra supplementation of phytase.
[4] No significant difference in doses of supplemented lactic acid was observed. Diets with extra supplementation of phytase (550 units/kg).

FERMENTED DIETS AND GROWTH PERFORMANCE OF PIGS

Fermented liquid co-products

Experiments focused on the addition of liquid co-products to pig diets are often digestibility trials (Edwards et al., 1986; Nicholson et al., 1988; Smits and Sebek, 1989; Smits and Jongbloed, 1996; Smits et al., 1998b), with a restricted weight range and number of pigs, and the addition of only one liquid co-product. Scholten et al. (1998) studied the effect of three fermented liquid co-products on the growth performance of growing-finishing pigs in the period of 25 to 113 kg liveweight (Table 6). The control diet consisted of compound feed mixed with water. The co-product diet consisted of the fermented liquid co-products, LWS, PSP and CW, and a complementary compound feed. The dry matter contributed by the co-products replaced 35% and 55% of the dry matter intake of the grower diet (25 to 45 kg liveweight) and the finisher diet (45 to 113 kg liveweight), respectively. Diets were prepared about one hour before feeding. Calculated energy content and nutrient levels in both diets were kept equal. Growing-finishing pigs fed co-product diet showed a higher average daily gain (768 versus 740 g/day; $P<0.05$) and

a better feed conversion ratio (2.58 versus 2.69; P<0.05) compared with the control diet (Table 6). The pH of the control and co-product diet ranged between 5.0 and 5.5 and between 4.0 and 4.5, respectively (Scholten, unpublished data).

Table 6. Performance of growing-finishing pigs fed non-fermented liquid compound diet (control) or liquid diet with fermented co-products liquid wheat starch, mashed potato steam peel and cheese whey (source: Scholten et al., 1998) .

	Control	Co-products	SEM[1]
Number of pigs	296	296	
Initial weight (kg)	25.1	25.1	
Final weight (kg)	111.3	113.4	
Daily gain (g)	740[a]	768[b]	4.7
Feed intake (kg/day)	1.99[a]	1.98[a]	0.01
Feed conversion ratio	2.69[a]	2.58[b]	0.02

[1] SEM = standard error of the mean (SAS).
[a,b] Data in a row with a different superscript differs P<0.05.

Fermented liquid compound diets

Data on the effect of dry compound diets, non-fermented liquid compound diets and fermented liquid compound diets on daily gain and feed conversion ratio (FCR) of weaned piglets were reviewed by Jensen and Mikkelsen (1998). They reviewed data from 17 trials and their summary is presented in Table 7. Fermented liquid compound diets were defined as "compound feed mixed with water and soaked for a period between 8 hours and several days", whereas non-fermented liquid compound diets were defined as "compound feed mixed with water and prepared immediately before feeding".

Table 7. Improvement (%) in daily gain and feed conversion ratio in experiments conducted to compare dry diets (DD), non-fermented (NWD) and fermented liquid compound diets (FWD) for weaned piglets (data adapted from Jensen and Mikkelsen, 1998)

	Number of trials	Daily gain		Feed conversion ratio[1]	
		Average ± SD[2]	Range	Average ± SD[2]	Range
NWD versus DD	10	12.3 ± 9.4	-7.5 to 34.2	-4.1 ± 11.8	-32.6 to 10.1
FWD versus NWD	3	13.4 ± 7.1	5.7 to 22.9	-1.4 ± 2.4	-4.8 to 0.6
FWD versus DD	4	22.3 ± 13.2	9.2 to 43.8	-10.9 ± 19.7	-44.3 to 5.8

[1] Negative (-) number means that feed conversion ratio is worse.
[2] Standard deviation.

On average, fermented liquid diets increased daily gain by 13.4% compared with non-fermented liquid diets, whereas no difference in FCR was observed (Table 7). The higher daily gain in combination with the increased FCR implies that fermented liquid compound diets increased feed consumption compared with non-fermented diets. It should be pointed out that for the experiments presented in Table 7, information about the stage of fermentation in the diet at feeding was lacking. This makes the interpretation of the responses obtained with both fermented and non-fermented diets between different studies difficult.

Feeding liquid diets to piglets has a negative effect on FCR compared with dry diets (Table 7). Russell et al. (1996) suggested that this negative effect is primarily a result of feed wastage. They observed that improvements in the trough design reduced the difference in FCR between dry and liquid diets. FCR for the dry-fed and liquid-fed piglets was 1.31 and 1.89 ± 0.05, respectively, in trial 1. In trial 2, improvements in the trough design resulted in FCR for the dry-fed and liquid-fed piglets of 1.37 and 1.44 ± 0.02, respectively (Russell et al., 1996). These results indicate the importance of developing a good trough design for feeding liquid diets. So far, however, little is known about the optimum trough design for liquid diets

From the information mentioned above, it seems that fermented liquid compound diets and fermented liquid co-products may improve daily gain, feed intake and feed conversion of pigs. More specific data on the effect of fermented diets on pig health is needed to allow more firm conclusion to be drawn.

POSSIBLE MODES OF ACTION OF FERMENTED DIETS

A successful application of fermented diets in pig nutrition requires an understanding of their modes of action. So far, the number of experiments conducted to examine this with fermented diets is scarce, but the environment of the gastrointestinal tract seems to be one of the places of action of fermented diets. Furthermore, the possible influence of fermented diets on physical activity of pigs may be a mode of action.

Stomach

It is generally considered that a low gastric pH inhibits the growth of undesirable microbes. At a low pH, the growth of harmful bacteria such as Coliforms and *Salmonella spp* is inhibited (Nout et al., 1989). In fermented diets, with a pH below 4.5, the number of Coliforms is reduced compared with non-fermented diets (Russell et al., 1996; Jensen and Mikkelsen, 1998). Feeding fermented liquid compound diets to weaned piglets reduced both gastric pH and the number of

Coliforms in the stomach compared with feeding non-fermented liquid compound diets (Mikkelsen and Jensen, 1997, 1998). Similarly, lactic acid supplemented pig diets (Thomlinson and Lawrence, 1981; Ratcliffe et al., 1986) or drinking water (Cole et al., 1968) reduce gastric pH and the number of Coliforms in the stomach. Feeding fermented diets resulted in an increase in lactic acid concentration in the stomach, whereas no differences in acetic acid, propionic acid and butyric acid were observed (Table 8). It is hypothesised that the reduced gastric pH and number of Coliforms in pigs fed fermented diets is caused by the high lactic acid concentration in the stomach.

Table 8. Concentrations of short chain fatty acids[1] and lactic acid in the gastrointestinal tract of piglets fed fermented liquid diet (FWD[2]) and piglets fed non-fermented liquid diets (NWD[3]). Data adapted from Mikkelsen and Jensen (1997)

	Lactic Acid		Acetic acid		Propionic acid		Butyric Acid	
	NWD	FWD	NWD	FWD	NWD	FWD	NWD	FWD
Stomach	37.3	81.7**	16.6	16.5	1.0	0.3	0.8	0.3
Small intestine I	7.7	26.3**	10.6	8.8	0.1	0.1	0.9	2.3
Small intestine II	13.7	24.5	10.2	9.1	0.6	0.5	0.0	4.7**
Small intestine III	38.0	32.7	11.7	9.8	0.1	0.0	0.2	2.7*
Small intestine IV	47.6	40.2	12.8	13.3	0.2	0.0	0.3	0.0
Caecum	2.3	2.9	73.8	93.0**	35.4	30.4	16.7	12.4*
Colon I	0.5	0.0	61.0	87.3***	28.8	30.7	16.5	14.2
Colon II	0.7	0.0	55.6	72.8**	26.2	25.7	17.3	13.2*
Colon III	1.0	0.0	60.1	69.9	24.6	25.6	15.6	13.5

1 mmol/kg digesta.
2 Seven animals.
3 Eight animals.
*,**,*** Significantly different from NWD: * P<0.05; ** P<0.01; *** P<0.001.

Coliforms are the main producers of toxic amines and ammonia (Dierick et al., 1986a,b), which are harmful for the gastrointestinal mucosa (Visek, 1972, 1978). Although the mechanism for the beneficial effect of anti-microbial growth promoters on growth performance and pig health is not very clear, the reduction of ammonia and/or toxic amines seems to be important (Dierick et al., 1986a; Health Council of the Netherlands, 1998). During fermentation, micro-organisms obtain energy from fermentation in the form of adenosine 5 triphosphate (ATP). Both the concentrations of ATP and lactic acid are indicators of microbial activity (Jensen and Mikkelsen, 1998). Feeding fermented diets reduced both the use of ATP (0.50 versus 0.99 mg ATP) and

1998). Feeding fermented diets reduced both the use of ATP (0.50 versus 0.99 mg ATP) and the level of lactic acid produced by microbial fermentation in the stomach (Jensen and Mikkelsen, 1998) compared with non-fermented liquid diets. Similar results were obtained when anti-microbial growth promoters or lactic acid was added to the diet of pigs (Jensen and Mikkelsen, 1998). Therefore, the reduction of the number of Coliforms seem to be one of the mechanisms whereby fermented diets may act.

Fermented diets may indirectly stimulate protein digestion by reducing gastric pH. First, a low gastric pH is needed to increase activity of pepsin (Taylor, 1959, 1962). Second, a low gastric pH reduces the rate of gastric emptying, thereby allowing more time for digestion to occur in the stomach (Mayer, 1994). So far, no experiments to study the effect of fermented diets on digestibility of protein and other nutrients have been conducted. The effect of acidified diets (organic acids) on apparent faecal digestibility has been the subject of a number of studies, reviewed by Partanen and Mroz (1999). They concluded that the addition of organic acids exerted a small positive influence on the apparent total tract digestibility of crude protein and energy. However, so far, no trials have been conducted in which the effect of a concomitant addition of lactic acid and acetic acid in the proportions commonly present in fermented diets has been assessed.

Pigs consume high numbers of lactic acid bacteria (about 5.0×10^9 cfu/g liquid diet) when fermented diets are fed (Mikkelsen and Jensen, 1998). Lactic acid bacteria are often used as probiotics (Vanbelle et al., 1990), which are defined as "live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance" (Fuller, 1989). In general, the effects of adding probiotics to (dry) diets on growth performance and pig health are inconsistent (as reviewed by Vanbelle et al., 1990; Nousianen and Setälä, 1993; Stavric and Komegay, 1995), and exact modes of action remain unclear (Nousiainen and Setälä, 1993). The studies with probiotics added to dry diets cannot be compared directly with studies in which fermented diets are fed. In fermented diets, both the bacteria and their fermentation products are present, whereas in dry diets, pigs consume only the bacteria and the results of their metabolism appear in the gastrointestinal tract of the animal. There is also a huge difference in the number of lactic acid bacteria and their activity; in fermented diets the bacteria grow rapidly, whereas in dry diets they have to be reaccelerated. Therefore, the specific contribution of lactic acid bacteria to the favourable results after feeding fermented diets is difficult to answer.

Small Intestine

Lactic acid and formic acid are produced in a high amounts by microbes in the small intestine (Jensen, 1998). Feeding fermented diets to piglets significantly reduced the level of ATP and microbial production of lactic acid and formic acid in the small intestine (Jensen and Mikkelsen, 1998) compared with non-fermented diets. As in the stomach, fermented diets reduce microbial activity in the small intestine, in particular the activity of Coliforms (Jensen and Mikkelsen, 1998). Despite the lower production of lactic acid in the small intestine, the absolute lactic acid concentration in the small intestine was higher when piglets were fed fermented diets, instead of non-fermented diets (Table 8). This implies that a part of the ingested lactic acid of the fermented diet is reaching the small intestine.

Although only significant in the first part of the small intestine, a higher pH was observed in the entire small intestine of pigs fed a fermented diet (Mikkelsen and Jensen, 1997) compared to pigs fed a non-fermented diet. This may result from a stimulation of the secretion of pancreatic juice, in particular bicarbonate, by the high concentration of lactic acid in the digesta reaching the small intestine. Both lactic acid and short-chain fatty acids (SCFA) elevate pancreatic secretion in pigs (Harada et al., 1986; Sano et al., 1995). An increase ($P<0.05$) in the volume as well as the protein content of pancreatic juice was observed in weaned piglets fed a dry diet supplemented with 25 g lactic acid/kg diet (Thaela et al., 1998), whereas trypsin, chymotrypsin and bicarbonate were numerically, but not statistically, higher. It should be pointed out that the lactic acid supplemented diet was fed for only one week. Moreover, the dose fed was much lower than that normally found in fermented diets (Table 4).

Weaning of piglets is often associated with villous shortening and crypt deepening in the small intestine (Kenworthy, 1976; Hampson, 1986; Nabuurs, 1991; Makkink, 1993; Van Beers-Schreurs, 1996), and is generally associated with a reduced digestive capacity and a reduced ability to absorb nutrients (reviewed by Pluske et al., 1997). Feed intake directly after weaning seem to prevent villous shortening (Makkink, 1993; Van Beers-Schreurs, 1996; Pluske et al., 1996a, 1996b, 1997). Feed intake can be increased by feeding fermented liquid diets (Russell et al., 1996; Mikkelsen and Jensen, 1997) or lactic acid supplemented diets (Cole et al., 1968; Roth et al., 1993; Jongbloed and Jongbloed, 1996). It is questionable, however, if feed intake per se prevents villous atrophy or that this response is mediated by SCFA and/or lactic acid. It is well known that SCFA are trophic for intestinal epithelial proliferation in rats (Sakata, 1987; Lupton and Kurtz, 1993; Frankel et al., 1994) and pigs (Sakata et al., 1995). This is supported by the fact that villous height in pigs is correlated positively with luminal level of SCFA, in particular butyric acid (Gálfi and Bokori, 1990; Nousiainen, 1991). Feeding fermented diets to weaned piglets results in higher ($P<0.05$; Table 8) butyric concentrations in the middle part of

the small intestine compared with non-fermented diets (Mikkelsen and Jensen, 1997). It may be suggested that fermented diets may be beneficial for mucosal structure. However, so far, no information is available about the effect of fermented diets on mucosal structure.

Large Intestine

Mikkelsen and Jensen (1997) observed a change in the proportions of acetic acid : propionic acid : butyric acid levels in the large intestine between fermented and non-fermented diets. Feeding fermented diets results in a higher acetic acid and lower butyric acid concentration compared with non-fermented diets (Table 8). Similarly, the addition of formic acid to dry diets shift the proportion of SCFA to higher proportions of acetic acid at the expense of butyric acid and/or propionic acid (Eidelsburger et al., 1992; Roth et al., 1992). This may be due to a shift in microbial population and, consequently, a shift in fermentation acids produced by microbes. Mikkelsen and Jensen (1997) found that feeding fermented diets to piglets resulted in a lower number of Coliforms in the large intestine. However, it should be pointed out that the concentrations of SCFA in the lumen do not indicate anything about the production and absorption of SCFA. Another possibility is that there is a shift in nutrients available for the microbes in the large intestine. More specific studies on the impact and the possible modes of action of the shift in microbial population and SCFA production and/or absorption in the large intestine is needed.

Physical activity

SCFA are absorbed through the gut wall and contribute essentially to the maintenance requirements of the gut (Just et al., 1983; Rérat et al., 1987). Experimental data suggest that SCFA produced in the large intestine may be related to physical activity of pigs. Several studies indicate that NSP-rich diets reduce physical activity of growing-finishing pigs (Schrama et al., 1996, 1998) and sows (Brouns et al., 1994; Matte et al., 1994). Schrama et al. (1997) reported that the effect of NSP-rich diets on physical activity seemed to be related to a stimulation of microbial fermentation in the gastrointestinal tract rather than gut fill. They suggest that an altered microbial population and specific effects of fermentation products (SCFA) may contribute to this response on physical activity. Brooks and Murray (unpublished data) observed significant differences in the behaviour of pigs fed fermented or non-fermented diets. Pigs fed a liquid fermented diet spent more time sleeping/resting than pigs fed a dry diet. Since less energy is used for physical activity, this may be an explanation for the higher daily gain when fermented diets are fed.

CONCLUDING REMARKS AND PERSPECTIVES

Liquid co-products and liquid compound diets ferment during storage and as a consequence changes in chemical and microbial properties occur. The stage of fermentation may be one of the factors contributing to the observed variability in chemical composition and different responses of pigs on feeding fermented diets. More detailed information about these changes and the effects on feeding value and other properties is needed.

Based on a limited number of experiments, fermented co-products and fermented compound diets may improve feed intake, daily gain and feed conversion ratio. Since fermented diets contain high levels of organic acids, they may be a potential alternative for the prophylactic use of anti-microbial growth promoters in pig diets. More studies are needed to elucidate the effects of fermented diets, including effects on pig health.

Fermented diets seem to reduce gastric pH, reduce the microbial activity and shifts the microbial population in the gastro-intestinal tract. Furthermore, it is hypothesized that fermented diets may stimulate pancreatic secretion and positively influence villous architecture. These factors may contribute to an improved digestion and absorption of nutrients when fermented diets are fed. In addition, it is hypothesized that fermented diets may reduce physical activity, which may be an explanation for the better growth performance. More specific studies are needed to elucidate the modes of action, which will stimulate more successful application of fermented diets in pig nutrition.

ACKNOWLEDGEMENTS

We wish to thank Prof. P.H. Brooks from the University of Plymouth in the United Kingdom for critically reading the manuscript.

REFERENCES

Beers-Schreurs, H.G.M. van, 1996. The changes in the function of the large intestine of weaned pigs. PhD-thesis, Universiteit Utrecht, Faculteit der Diergeneeskunde, The Netherlands. 149 pp.

Brouns, F., Edwards, S.A., 1994. Effect of dietary fibre and feeding system on activity and oral behaviour of group housed gilts. Appl. Anim. Behav. Sci. 39, 215.

Chaubert, C., 1995. Die Nebenprodukte der Lebensmittelherstellung. Agrarforschung 2, 49-52.

Cole, D.J.A., Beal, R.M., Luscombe, J.R., 1968. The effect on performance and bacterial flora of lactic acid, propionic acid, calcium propionate and calcium acrylate in the drinking water of weaned pigs. Vet. Rec. 83, 459-464.

CVB, 1997. Centraal Veevoederbureau. Berekeningswijze van de netto energiewaarde van voedermiddelen voor varkens (in Dutch). CVB-reeks nr. 21. Lelystad, The Netherlands.

CVB, 1998. Central Veevoederbureau. Veevoedertabel. Lelystad, The Netherlands.

Dahl, J., 1997. Feed-related risk factors for sub-clinical salmonella infections. (in Danish). Veterinærinformation, December 1997, 17-20.

Dierick, N.A., Vervaeke, I.J., Decuypere, J.A., Henderickx, H.K., 1986a. Influence of the gut flora and of some growth-promoting feed additives on nitrogen metabolism in pigs. I. Studies in vitro. Livest. Prod. Sci. 14, 161-176.

Dierick, N.A., Vervaeke, I.J., Decuypere, J.A., Henderickx, H.K., 1986b. Influence of the gut flora and of some growth-promoting feed additives on nitrogen metabolism in pigs. II. Studies in vivo. Livest. Prod. Sci. 14, 177-193.

Edwards, S.A., Fairbairn, C.B., Capper, A.L., 1986. Liquid potato feed for finishing pigs: feeding value, inclusion rate and storage properties. Anim. Feed Sci. Technol. 15, 129-139.

Eidelsburger, U., Kirchgessner, M., Roth, F.X., 1992. Zum Einfluß von Ameisensäure, Calciumformiat und Natriumhydrogencarbonat auf pH-wert, Trockenmassegehalt, konzentration an Carbonsauren und ammoniak in verschiedenen Segmenten des Gastrointestinaltraktes. J. Anim. Physiol. Anim. Nutr. 68, 20-32.

Frankel, W.L., Zhang, W., Singh, A., Klurfeld, D.M., Don, S., Sakata, T., Modlin, I., Rombeau, J.L., 1994. Mediation of the trophic effects of short-chain fatty acids on the rat jejunum and colon. Gastroenterology 106, 375-380.

Fuller, R., 1989. A review: probiotics in man and animals. J. Appl. Bacteriol. 66, 365-378.

Gàlfi, P., Bokori, J., 1990. Feeding trial in pigs with a diet containing sodium n-butyrate. Acta Veterinaria Hungarica 38, 3-17.

Geary, T.M., Brooks, P.H., Morgan, D.T., Campbell, A., Russell, P.J., 1996. Performance of weaner pigs fed ad libitum with liquid feed at different dry matter concentrations. J. Sci. Food Agric. 72, 17-24.

Hampson, D.J., 1986. Alterations in piglet small intestinal structure at weaning. Res. Vet. Sci. 40, 32-40.

Harada, E., Niiyama, M., Syuto, B., 1986. Comparison of pancreatic exocrine secretion via endogenous secretin by intestinal infusion of hydrochloric acid and monocarboxylic acid in anesthetized piglets. Japanese J. of Physiol. 36, 843-858.

Health Council of the Netherlands: Committee on Antimicrobial growth promotors. Antimicrobial growth promotors. Rijswijk: Health Council of the Netherlands, 1998. Publication no. 1998/15, 113 pp.

Jensen, B.B., 1998. The impact of feed additives on the microbial ecology of the gut in young pigs. J. of Anim. Feed Sci. 7, 45-64.

Jensen, B.B., Mikkelsen, L.L., 1998. Feeding liquid diets to pigs. In: Garnsworthy P.C., Wiseman, J., (Eds.), Recent Advances in Animal Nutrition. Nottingham University Press, Loughborough, U.K., pp. 107-126.

Jongbloed, A.W., Jongbloed, R., 1996. The effect of organic acids in diets for growing pigs on enhancement of microbial phytase efficacy. Report ID-DLO No. 96009, Lelystad, The Netherlands.

Just, A., Fernández, J.A., Jorgensen, H., 1983. The net energy value of diets for growth in pigs in relation to the fermentative process in the digestive tract and the site of absorption of the nutrients. Livest. Prod. Sci. 10, 171-186.

Kenworthy, R., 1976. Observations on the effects of weaning in the young pig. Clinical and histopathological studies of intestinal function and morphology. Res. Vet. Sci. 2-1, 69-75.

Lupton, J.R., Kurtz, P.P., 1993. Relationship of colonic luminal short-chain fatty acids and pH to in vivo cell proliferation in rats. American Institute of Nutrition, 1522-1530.

Makkink, C.A., 1993. Of piglets, dietary proteins and pancreatic proteases. PhD-thesis, Department of Animal Nutrition, Agricultural University Wageningen, The Netherlands, 168 pp.
Matte, J.J., Robert, S., Girard, C.L., Farmer, C., Martineau, G.-P., 1994. Effect of bulky diets based on wheat bran or oat hulls on reproductive performance of sows during their first two parities. J. Anim. Sci. 72, 1754-1760.
Mayer, E.A., 1994. The physiology of gastric storage and emptying. In: Johnson, L.R., Alpers, D.H., Christensen, J., Jacobson, E.D., Walsh, J.H., (Eds.), Physiology of the gastrointestinal tract. Third edition. Volume 1. New York: Raven Press. pp. 929-976.
Mikkelsen, L.L., Jensen, B.B., 1997. Effect of fermented liquid feed (FLF) on growth performance and microbial activity in the gastrointestinal tract of weaned piglets. In: Laplace, J.P., Fevrier, C., Barbeau, A, (Eds.), Digestive Phyiology in Pigs, E.A.A.P. publication no. 88. Saint Malo, France, May 26-28, p. 639-642.
Mikkelsen, L.L., Jensen, B.B., 1998. Performance and microbial activity in the gastrointestinal tract of piglets fed fermented liquid feed at weaning. J. of Anim. Feed Sci. 7, 211-215.
Moreau, R., Quémeré, P., Carlier, J.C., 1992. Utilisation de sous-produits agro-industriels dans l'alimentation du porc: 1- Pratiques et résultats technico-économiques dans le Nord de la France (in French, with Englisch summary). Journées Rech. Porcine en France 24, 143-150.
Müller, G., 1996. Die Nutzung von Nebenprodukten in der Tierernährung aus ökonomischer Sicht. Proceedings Workshop Unkonventionelle Futtermittel, 10/11 April 1996, Braunschweig, p. 37-46.
Nabuurs, M., 1991. Etiologic and pathogenic studies on postweaning diarrhea. PhD-thesis, Centraal Diergeneeskundig Instituut, Lelystad, The Netherlands. 131 pp.
Nicholson, J.W.G., Snoddon, P.M., Dean, P.R., 1988. Digestibility and acceptability of potato steam peel by pigs. Can. J. Anim. Sci. 68, 233-239.
Nousiainen, J., 1991. Comparative observations on selected probiotics and olaquindox used as feed additives for piglets around weaning - 2. Effect on villus height and crypt depth in the jejunum, ileum, caecum and colon. J. Anim. Physiol. Anim. Nutr. 66, 224-230.
Nousiainen, J., Setälä, J., 1993. Lactic acid bacteria as animal probiotics. In: Salminen, S., Wright, A. von, (Eds.), Lactic acid bacteria, Marcel Dekker, New York, US, pp. 315-356.
Nout, M.J.R., Rombouts, F.M., Havelaar, A., 1989. Effect of accelerated natural lactic fermentation of infant food ingedrients on some pathogenic microorganisms. International Journal of Food Microbiology 8, 351-361.
Partanen, K., Mroz, Z., 1998. Organic acids for performance enhancement in pig diets. Nutr. Res. Rev. 12, 1-30.
Pluske, J.R., Williams, I.H., Aherne, F.X., 1996a. Maintenance of villous height and crypt depth in piglets by providing continuous nutrition after weaning. Animal Science 62, 131-144.
Pluske, J.R., Williams, I.H., Aherne, F.X., 1996b. Villous height and crypt depth in piglets in response to increases in the intake the intake of cows' milk after weaning. Anim. Sc. 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.
Prescott, L.M., Harley, J.P., Klein, D.A., 1996. Microbiology. WCB Publishers. 935 pp.
Ratcliffe, B., Cole, C.B., Fuller, R., Newport, M.J., 1986. The effect of yoghurt and milk fermented with a porcine intestinal strain of *Lactobacillus reuteri* on the performance and gastrointestinal flora of pigs weaned at two days of age. Food Microbiology 3, 203-211.
Rérat, A., Fiszlewicz, M., Giusi, A., Vaugelade, P., 1987. Influence of meal frequency on postprandial variations in the production of absorption of volatile fatty acids in the digestive tract of conscious pigs. J. Anim. Sci. 64, 448-456.
Roth, F.X., Kirchgessner, M., 1988. Zum Einsatz von Essigsäure in der Ferkelfütterung. Landwirtsch. Forsch. 41, 253-258.

Roth, F.X., Eidelsburger, U., Kirchgessner, M., 1992. Zum Einfluß von Fumarsäure, Salzsäure, Natriumformiat, Tylosin und Toyocerin auf pH-wert, Trockenmassegehalt, Konzentration an Carbonsauren und Ammoniak in verschiedenen Segmenten des Gastrointestinaltraktes. J. Anim. Sci. 68, 93-103.

Roth, F.X., Kirchgessner, M., Eidelsburger, U., 1993. Zur Nutritiven Wirksamkeit von Michsäure in der Ferkelaufzucht. Agribiol. Res. 46, 229-239.

Russell, P.J., Geary, T.M., Brooks, P.H., Campbell, A., 1996. Performance, water use and effluent output of weaner pigs fed ad libitum with either dry pellets or liquid feed and the role of microbial activity in the liquid feed. J. Sci. Food Agric. 72, 8-16.

Sakata, T., 1987. Stimulatory effect of short-chain fatty acids on epithelial cell proliferation in the rat intestine: a possible explanation for trophic effects of fermentable fibre, gut microbes and luminal trophic factors. Br. J. Nutr. 58, 95-103.

Sakata, T., Adachi, M., Hashida, M., Sato, N., Kojima, T., 1995. Effect of n-butyric acid on epithelial cell proliferation of pig colonic mucosa in short-term culture. Dtsch. Tierärtzl. Wschr. 102, 163-164.

Sano, H., Nakmura, E., Takahashi, H., Terashima, Y., 1995. Plasma insulin and glucagon responses to acute challenges of acetate, propionate, n-butyrate and glucose in growing gilts (Sus scrofa). Comparative Biochemistry and Physiology 110A, 375-378.

Scholten, R., Verdoes, N., 1997. The Dutch benefit from a recycling role. Pigs 13-2, 16-17.

Scholten, R.H.J., Rijnen, M.M.J.A., 1998. The use of liquid by-products. A review. (in Dutch, with Englisch summary). Proefverslag P1.210. Praktijkonderzoek Varkenshouderij, Rosmalen, The Netherlands.

Scholten, R.H.J., Hoofs, A.I.J., Verdoes, N., 1997. By-products related to performance and environmental characteristics at growing/finishing pigs. (in Dutch, with Englisch summary). Proefverslag P1.187. Praktijkonderzoek Varkenshouderij, Rosmalen, The Netherlands.

Scholten, R.H.J., Peet-Schwering, C.M.C. van der, Hartog, L.A. den, Vesseur, P.C., Verstegen, M.W.A., 1998. Effect of liquid by-products on performance and health of pigs. Paper N.2.2 presented at 49th Annual Meeting of the European Association for Animal Production, Commission on Animal Nutrition, 24th-27th August, Warsaw Poland.

Schrama, J.W., Verstegen, M.W.A., Verboeket, P.H.J., Schutte, J.B., Haaksma, J., 1996. Energy metabolism in relation to physical activity in growing pigs as affected by type of dietary carbohydrate. J. Anim. Sci. 74, 2220-2225.

Schrama, J.W., Heetkamp, M.J.W., Bakker, G.C.M., Vught, P. van, Verstegen, M.W.A., 1997. Invloed van fermentatie van koolhydraten en vulling van maagdarmkanaal op de activiteit in relatie tot de energiestofwisseling bij vleesvarkens. (in Dutch). Landbouwuniversiteit Wageningen, Vakgroepen Veehouderij en Veevoeding, The Netherlands. 39 pp.

Schrama, J.W., Bosch, M.W., Verstegen, M.W.A., Vorselaars, A.H.P.M., Haaksma, J., Heetkamp, M.J.W., 1998. The energetic value of nonstarch polysaccharides in relation to physical activity in group-housed, growing pigs. J. Anim. Sci. 76, 3016-3023.

Shelef, L.A., 1994. Antimicrobial effects of lactates: a review. J. Food Protect. 57, 445-450.

Siva, 1997. Kengetallenspiegel. Uitgave Siva Produkten. Wageningen. The Netherlands.

Smits, B., 1998a. Effect van bewaring en droge stofbepaling op de chemische samenstelling en op de mate van vervluchtiging van vluchtige bestanddelen in vochtrijke enkelvoudige diervoeders (in Dutch). Rapport ID-DLO no. 98.031. Instituut voor Dierhouderij en Diergezondheid, Lelystad, The Nederlands.

Smits, B., 1998b. Chemical composition, digestibility and nutritive value of high moisture by-products in pig nutrition. Paper N2.1 presented at 49th Annual Meeting of the European Association for Animal Production, Commission on Animal Nutrition, 24th-27th August, Warsaw Poland. EAAP.

Smits, B., Sebek, L.B.J., 1989. Verteerbaarheid en voederwaarde van BONDATAR: twee verteringsproeven (in Dutch, with Englisch summary). Rapport IVVO no. 201, Instituut voor Veevoedingsonderzoek, Lelystad. The Netherlands.

Smits, B., Jongbloed, R., 1996. Ileale en faecale verteerbaarheid en voederwaarde van twee partijen aardappelstoomschillen voor vleesvarkens (in Dutch). Rapport ID-DLO no. 96003. Instituut voor Dierhouderij en Diergezondheid, Lelystad, The Nederlands.

Smits, B., Gelder, A.H. van, Cone, J.W., 1996. Samenstelling koolhydraatfractie in partijen aardappelstoomschillen, ontsloten aardappelzetmeel, tarweindampconcentraat en tarwezetmeel (in Dutch). Rapport ID-DLO no 96.013. Instituut voor Dierhouderij en Diergezondheid, Lelystad, The Nederlands.

Stavric, S., Kornegay, E.T., 1995. Microbial probiotics for pigs and poultry. In: Biotechnology in Animal Feeds and Animal Feeding, pp. 205-231.

Taylor, W.H., 1959. Studies on gastric proteolysis. Biochem. J. 71, 627-632.

Taylor, W.H., 1962. Proteinases of the stomach in health and disease. Physiol. Rev. 42, 519-553.

Thaela, M.J., Jensen, M.S., Pierzynowski, S.G., Jakob, S., Jensen, B.B., 1998. Effect of lactic acid supplementation on pancreatic secretion in pigs after weaning. J. Anim. Feed Sci. 7, suppl. 1, 181-183.

Thomlinson, J.R., Lawrence, T.L.J., 1981. Dietary manipulation of gastric pH in the prophylaxis of enteric disease in weaned pigs: some field observations. Vet. Rec. 109, 120-122.

Vanbelle, M., Teller, E., Focant, M., 1990. Probiotics in animal nutrition: a review. Arch. Anim. Nutr. Berlin 40, 543-567.

Visek, W.J., 1972. Effects of urea hydrolysis on cell life-span and metabolism. Fed. Proc. Fed. Am. Soc. Exp. Biol. 31, 1178-1193.

Visek, W.J., 1978. The mode of growth promotion by antibiotics. J. Anim. Sci. 46, 1447-1469.

Wolf, P.J., van der, Bongers, J.H., Elbers, A.R.W., Franssen, F.M.M.C., Hunneman, W.A., Exsel, A.C.A., Tielen, M.J.M., 1999. Salmonella infections in finishing pigs: bacteriological herd prevalence, serogroup and antibiotic resistance of isolates and risk factors for infection. Vet. Microbiol. 67 (4), 263-275.

Chapter 2

FERMENTATION OF LIQUID CO-PRODUCTS AND LIQUID COMPOUND DIETS: PART 1. EFFECTS ON CHEMICAL COMPOSITION DURING A 6-DAY STORAGE PERIOD

Ronald H.J. Scholten[1,*], Martin M.J.A. Rijnen[2], Johan W. Schrama[3], Huug Boer[2], Peter C. Vesseur[1], Leo A. den Hartog[1], Carola M.C. van der Peet-Schwering[1], Martin W.A. Verstegen[2]

[1] Research Institute for Animal Husbandry, Lelystad, The Netherlands

[2] Wageningen Institute of Animal Sciences, Animal Nutrition Group, Wageningen, The Netherlands

[3] Wageningen Institute of Animal Sciences, Fish Culture and Fisheries Group, Wageningen, The Netherlands

[*] Present address: Beuker Vochtrijke Diervoeders B.V., Doetinchem, The Netherlands

Published in:

Reprinted from Journal of Animal Physiology and Animal Nutrition 85 (2001) 111-123

with permission of Blackwell Wissenschafts-Verlag

ABSTRACT

The effects of a 6-day storage period on changes in dry matter, crude ash, crude protein, true protein, crude fat, starch, soluble starch, sugar and lactose of three liquid co-products and two liquid compound diets were studied. The three liquid co-products studied were: liquid wheat starch (**LWS**), mashed potato steam peel (**PSP**) and cheese whey (**CW**), and the two liquid compound diets were: liquid grower diet (**LGD**) and liquid finisher diet (**LFD**). The loss of corrected dry matter after a 6-day storage, expressed in relation to the initial content, was 1.9, 6.2, 9.6, 4.6 and 4.2% for LWS, PSP, CW, LGD and LFD, respectively. During storage, the total amount of starch decreased 2.7, 24.0, 28.1 and 33.3% for LWS, PSP, LGD and LFD, respectively. The total amount of lactose decreased 23.5% for CW. The gross energy value of the products did not change remarkably during the 6-day storage period; gross energy losses being less than 3% of the initial gross energy content.

INTRODUCTION

In Europe, several million tons of liquid co-products from human food industries are recycled in pig nutrition annually. In particular, starch and sugar rich co-products like liquid wheat starch (LWS), mashed potato steam peel (PSP) and cheese whey (CW) are used in pig nutrition (Scholten et al., 1999a). These co-products ferment readily during storage, and consequently pigs receive diets containing products originating from fermentation (Smits, 1998; Scholten et al., 1999b). Similarly, liquid compound diets, consisting of compound feed and water, ferment while they are soaked for at least 8 to 24 hours (Russell et al., 1996; Jensen and Mikkelsen, 1998). Liquid diets containing fermentation products seem to improve growth performance of weaned piglets and growing-finishing pigs (Jensen and Mikkelsen, 1998; Scholten et al., 1999b).

Fermentation is a dynamic process during which in particular starch and sugars are transformed by micro-organisms into lactic acid, volatile fatty acids (**VFA**), alcohol and CO_2 (Prescott et al., 1996). This will lead to changes in chemical composition as well as physical properties. Therefore, storage time may be an important determinant for the magnitude of changes in chemical composition of liquid feedstuffs. In return, these changes may affect the nutritional value of these feedstuffs. The aim of the present study was to quantify chemical changes in time of LWS, PSP, CW, a liquid grower diet (LGD) and a liquid finisher diet (LFD) during a 6-day storage period.

MATERIALS AND METHODS

Products

Three different liquid co-products and two liquid compound diets were studied: liquid wheat starch, mashed potato steam peel, cheese whey, liquid grower diet and liquid finisher diet. The composition of both compound diets is given in Table 1.

Table 1. Composition (g/kg) of the grower feed and finisher feed used for liquid grower diet (LGD) and liquid finisher diet (LFD)

	LGD	LFD
Wheat	125.0	34.0
Barley	175.0	-
Peas	68.0	123.0
Rape seed, extracted	81.0	175.0
Soya beans, extracted	160.0	88.0
Sunflower seed, extracted	50.0	21.0
Wheat middlings	36.0	48.0
Maize glutenfeed	-	27.0
Molasses, beet	20.0	30.0
Tapioca	233.5	400.0
Fat	24.5	42.0
Limestone	10.0	3.3
Salt	2.0	1.7
Rukanaphos	5.7	1.8
Premix	5.0[1]	2.5[2]
Phytase	2.3	2.4
Methionine-50%	0.6	0.3
Lysine-40%	1.4	-

[1] No acids; 50 mg olaquindox; 160 mg Cu; 8000 IE Vit A; 1500 IE Vit D3; 30 IE Vit E.
[2] No acids; 45 mg zincbacitrine; 25 mg Cu; 6000 IE Vit A; 1200 IE Vit D3; 25 IE Vit E.

The experiment consisted of a storage period of six days (144 hours), which was repeated three times. Approximately one hour before the start of each storage period, fresh batches of LWS, PSP and CW were delivered straight from the production plants. The grower and finisher compound feed, obtained from a commercial feed supplier, were both pelleted from the same batch of feedstuffs. One hour before the start of each storage period, the grower and finisher

diets were mixed with water in a water to feed ratio of 2.5:1. Both LGD and LFD were soaked and stirred continuously for one hour, before starting the storage period (T_0).

Storage

The results of a preliminary study showed that LWS, PSP and CW reached a steady pH of 3.5 to 3.7 after four days of storage and LGD and LFD a steady pH of 3.8 to 4.0 after five days of storage (data not shown). Therefore, in the present study, a storage period of six days (144 hours) was sufficient to ensure a stationary pH before the end of each storage period.

The five products (i.e., 45 kg of each product) were stored separately in 50 liter lockable PVC tanks for six days. The storage tanks were provided with a stirrer and lockable PVC opening, to enable sampling. All products were stirred automatically for one minute every two hours. PSP was not stirred as no separation was expected due to its high viscosity and because in practice this product is also not stirred. Samples (500 ml) were removed at 0, 12, 24, 36, 48, 72, 96 and 144 hours after the start of each storage period (T_0, T_{12}, T_{24}, T_{36}, T_{48}, T_{72}, T_{96} **and** T_{144}, respectively). Immediately before sampling, LWS, CW, LGD and LFD were additionally stirred for one minute. After collection, samples were immediately stored at -20°C. A cleaned PVC pipe (Ø 40 mm) with a wire on the inside and an attached rubber stopper at the end, was used for sampling. This sampling technique allowed a lengthwise sample of the product.

The storage tanks were placed in a temperature-controlled room at 23 ± 1 °C. Within each replicate, products were randomly assigned to a storage tank (number 1 to 5). The tanks were cleaned with hot water, disinfected, and dried between replicates.

Chemical analyses

Samples were analysed in duplicate for dry matter (DM), crude ash (ASH), crude protein (CP), true protein (TP), crude fat (CFAT), starch, soluble starch, sugars, lactose, pH, acid binding capacity, lactic acid, volatile fatty acids (VFA) and ethanol. The data on DM, ASH, CP, TP, CFAT, starch, soluble starch, sugar and lactose are presented in this paper.

The DM content was determined in all samples, whereas ASH, TP, CP and CFAT concentrations were determined in samples derived at T_0 and T_{144}. The starch, soluble starch and sugar content were only determined in LWS, PSP, LGD and LFD at T_0, T_{36}, T_{72} and T_{144}. The lactose concentration was only determined in CW samples.

The starch concentration was determined according to NIKO-MEMO 93-302, as described by Goelema et al. (1998); reducing sugars and soluble starch were detemined according to Smits et al. (1994). The lactose concentration was determined using High Performance Liquid Chromatography (**HPLC**; method Borculo Domo Ingredients, The Netherlands); CFAT, DM, ASH

and nitrogen were determined according to standard methods ISO/DIS 6492 (ISO, 1996), ISO 6469 (ISO, 1983), ISO 5984 (ISO, 1978) and ISO 5983 (ISO, 1979), respectively. Multiplying the nitrogen content by 6.38 (CW) or 6.25 (LWS, PSP, LGD, LFD) calculated CP. TP was determined according to the method of Stutzer and Barnstein (1900).

Calculations

The standard method for determining the DM content, allows the volatilisation of significant quantities of VFA, lactic acid and alcohol during the oven drying process. Consequently, the DM content in products with high levels of VFA, lactic acid and ethanol is underestimated. Most liquid co-products contain high levels of volatile components. Therefore, a corrected DM (cDM) concentration was calculated using the formula of CVB (1999):

Formula (1) **cDM** (g/kg) = DM (g/kg) + 0.08 × lactic acid content (g/kg) + 0.50 × VFA content (g/kg) + 1.00 × ethanol content (g/kg)

in which DM, lactic acid, VFA and ethanol are in fresh material.

The total levels of VFA+lactic acid+ethanol at T_0 and T_{144} are summarized in Table 5, whereas the detailed information about the individual components was taken from Scholten et al. (2001).

The initial content of the storage tanks was 45.0 kg. To obtain information on the weight changes of the products during storage, it was assumed that fermentation did not affect the total ASH content. Therefore, the total weight of the product at T_{144} was calculated as:

Formula (2) Total weight T_{144} (kg) = (ASH T_0 / ASH T_{144}) * 45.0 kg

To obtain information on the changes of the total amount of cDM (kg) and moisture (kg), the following formulas were used:

Formula (3) Total weight cDM (kg) = Total weight (kg) * (cDM / 1000)

Formula (4) Total weight moisture (kg) = Total weight (kg) – Total weight cDM (kg)

The gross energy (**GE**) content of the liquid feeds was calculated by multiplying the total amount of each nutrient (g in 45 kg product) by the heat of combustion (kJ/g). The following values for heat of combustion were used: CP 23.6 kJ/g, CFAT 39.6 kJ/g, starch 17.5 kJ/g, soluble starch 17.5 kJ/g, sugar 15.7 kJ/g, lactose 16.5 kJ/g, lactic acid 15.2 kJ/g, acetic acid 14.6 kJ/g, formic acid 5.7 kJ/g, propionic acid 20.8 kJ/g, (iso)butyric acid 24.9 kJ/g, (iso)valeric acid 27.0 kJ/g and ethanol 29.8 kJ/g (Weast, 1968).

Statistical analyses

All statistical procedures were carried out using the General Linear Model procedure of SAS (SAS, 1994). Preliminary analyses showed no differences between replicates. Therefore, 'replicates' was not used as factor in the model. The model used was:

$$Y_i = \mu + P_i + e_i$$

where Y_i = dependent variable; μ = overall mean; P_i = product (i = 1, .., 5); e_i = error term. The same model was used to analyse if the change in time was different from zero (probability > |T| H0: LSMEAN = 0; SAS, 1994).

RESULTS

Initial chemical composition

At the start of the storage period (T_0), the content of dry matter (DM) varied between 69.5 (CW) and 253.3 g/kg (LFD) (Table 2). The total level of starch, soluble starch plus sugar was 538.2, 619.7, 455.6 and 440.7 g/kg DM for LWS, PSP, LGD and LFD, respectively (Table 2). CW contained 680.9 g of lactose per kg DM (Table 2).

Table 2. Initial chemical composition (g/kg DM)[1] of liquid wheat starch (LWS), mashed potato steem peel (PSP), cheese whey (CW), liquid grower diet (LGD) and liquid finisher diet (LFD)

	LWS	PSP	CW	LGD	LFD
Dry matter (g/kg)	224.7	139.8	69.5	249.5	253.3
Crude Protein	134.9	128.8	145.6	209.7	190.8
Crude Fat	32.5	9.9	18.6	48.2	69.4
Crude Ash	27.5	52.9	100.9	68.0	68.0
Starch	323.3	603.7	n.d.[2]	373.9	347.7
Soluble starch	90.2	7.0	n.d.	11.0	7.0
Sugars	124.7	9.0	n.d.	71.7	86.0
Lactose	n.d.	n.d.	680.9	n.d.	n.d.

[1] Average of 3 batches.
[2] Not determined.

Dry matter and corrected dry matter

The dry matter (DM) and corrected dry matter (cDM) contents at the start (T_0) and after 144 hours of storage (T_{144}) differed between the products ($P<0.05$), except for LGD and LFD ($P>0.10$; Tables 3a+3b).

Table 3a. Initial content, final content and changes during a 6-day storage period in dry matter content (g/kg) of liquid wheat starch (LWS), mashed potato steam peel (PSP), cheese whey (CW), liquid grower diet (LGD) and liquid finisher diet (LFD)

	Dry Matter					
Time	LWS	PSP	CW	LGD	LFD	SEM
T_0	224.7^a	139.8^b	69.5^c	249.5^d	253.3^d	3.42
T_{144}	219.2^a	129.0^b	61.2^c	228.4^a	229.5^a	5.06
$T_0 - T_{12}$	0.6	3.6	0.9	1.1	0.4	
$T_0 - T_{24}$	0.6	3.9**	2.0$^{\#}$	2.7*	1.2	
$T_0 - T_{36}$	1.7	6.3***	2.7$^{\#}$	5.1**	4.4**	
$T_0 - T_{48}$	3.1	4.6*	3.5$^{\#}$	2.4	3.0	
$T_0 - T_{72}$	3.9	6.5*	5.5*	6.4*	8.5**	
$T_0 - T_{96}$	4.3	9.0*	7.3$^{\#}$	9.1*	12.7**	
$T_0 - T_{144}$	5.5	10.8$^{\#}$	8.3	21.1**	23.8**	

^{a,b,c,d} Mean values at time 0 (T_0) or time 144 (T_{144}) within a row with a different superscript are different: $P<0.05$.

*, **, *** Mean of the time changes differs from zero: $^{\#}$ $P<0.10$; * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

At the start of the storage period, the DM content was 7.7, 1.4, 1.2, 0.6 and 0.6 g/kg lower compared with the cDM content of LWS, PSP, CW, LGD and LFD, respectively (Tables 3a+3b). At the end of the 144 hours of storage, the difference between DM and cDM increased to 8.8, 3.4, 2.7, 10.1 and 13.9 g/kg for LWS, PSP, CW, LGD and LFD, respectively (Tables 3a+b).

In both LGD and LFD the decrease in DM and cDM content after 144 hours of storage was significant ($P<0.01$ and $P<0.05$, respectively; Tables 3a+b). In PSP, the decrease in DM and cDM was significant ($P<0.10$), whereas no decreases in DM and cDM contents in LWS and CW after 144 hours storage were observed ($P>0.10$; Tables 3a+b).

Table 3b. Initial content, final content and changes during a 6-day storage period in dry matter content (g/kg) of liquid wheat starch (LWS), mashed potato steam peel (PSP), cheese whey (CW), liquid grower diet (LGD) and liquid finisher diet (LFD)

	Corrected Dry Matter					
Time	LWS	PSP	CW	LGD	LFD	SEM
T_0	232.4^{a}	141.2^{b}	70.7^{c}	250.1^{d}	253.9^{d}	3.57
T_{144}	228.0^{a}	132.4^{b}	63.9^{c}	238.5^{ad}	243.3^{d}	4.56
$T_0 - T_{12}$	0.5	3.0$^{\#}$	0.8	1.1	0.5	
$T_0 - T_{24}$	0.7	2.7*	1.6	2.5*	1.3	
$T_0 - T_{36}$	1.2	5.0**	2.2$^{\#}$	3.6**	3.2*	
$T_0 - T_{48}$	2.8	3.2$^{\#}$	2.8	-1.2	0.1	
$T_0 - T_{72}$	3.4	5.1*	4.5$^{\#}$	1.0	4.6$^{\#}$	
$T_0 - T_{96}$	3.8	7.3*	6.0$^{\#}$	1.5	5.9$^{\#}$	
$T_0 - T_{144}$	4.4	8.8$^{\#}$	6.8	11.6*	10.6*	

^{a,b,c,d} Mean values at time 0 (T_0) or time 144 (T_{144}) within a row with a different superscript are different: P<0.05.

*, **, *** Mean of the time changes differs from zero: $^{\#}$ P<0.10; * P<0.05; ** P<0.01; *** P<0.001.

Crude ash, crude fat, and crude and true protein

Among the five products, there were differences in the contents of ASH, CFAT, CP and TP at both T_0 and T_{144} (P<0.05; Table 4), due to the chemical characteristics of the used products.

In all liquid feeds no significant changes in concentration of ASH, CFAT, CP and TP occurred during storage, except for the ASH content in LGD and CW (P<0.001 and P<0.05, respectively) and the CFAT content in LGD and LFD (P<0.01 and P<0.05, respectively; Table 4).

Table 4. Initial content, final content and changes during a 6-day storage period in crude ash, crude fat, crude protein and true protein content (g/kg) and ratio of true protein:crude protein in liquid wheat starch (LWS), mashed potato steam peel (PSP), cheese whey (CW), liquid grower diet (LGD) and liquid finisher diet (LFD)

	PRODUCT					
	LWS	PSP	CW	LGD	LFD	SEM
Crude ash						
T_0	6.40^a	7.47^a	7.17^a	17.00^b	17.27^b	0.493
T_{144}	6.40^a	7.40^a	6.83^a	17.07^b	18.10^b	0.438
$T_0 - T_{144}$	0.00	0.07	0.34*	-0.07	-0.83***	
Crude fat						
T_0	7.54^a	1.40^b	1.33^b	12.07^c	17.63^d	0.556
T_{144}	6.87^a	1.54^b	1.23^b	10.87^c	18.70^d	0.481
$T_0 - T_{144}$	0.67$^{\#}$	-0.14	0.10	1.20**	-1.07*	
Crude protein						
T_0	31.36^a	18.19^b	10.34^c	52.46^d	48.46^d	1.322
T_{144}	31.31^a	17.96^b	10.42^c	52.52^d	48.92^e	1.050
$T_0 - T_{144}$	0.05	0.23	-0.08	-0.06	-0.46	
True protein						
T_0	16.92^a	13.05^b	6.13^c	45.18^d	41.27^e	0.802
T_{144}	16.89^a	13.54^b	5.72^c	44.18^d	40.87^e	0.990
$T_0 - T_{144}$	0.03	-0.49	0.41	1.00	0.40	
Ratio true:crude protein						
T_0	0.54^a	0.72^b	0.58^a	0.86^c	0.85^c	0.014
T_{144}	0.54^a	0.75^b	0.54^a	0.84^c	0.83^c	0.015
$T_0 - T_{144}$	0.00	-0.04$^{\#}$	0.04$^{\#}$	0.02	0.02	

^{a,b,c,d} Mean values at time 0 (T_0) or time 144 (T_{144}) within a row with a different superscript are different: P<0.05.

*, **, *** Mean of time changes differs from zero: $^{\#}$ P<0.10; * P<0.05; ** P<0.01; *** P<0.001.

Carbohydrates

The concentrations of starch, soluble starch and sugar at both T_0 and T_{144} were different between LWS and PSP ($P<0.05$), whereas between LGD and LFD no differences were observed ($P>0.10$; Table 5). At T_{72} and T_{144} a significant decline in starch concentration of PSP, LGD and LFD was observed ($P<0.001$), whereas the starch level of LWS remained stable ($P>0.10$; Table 5).

In LWS the initial soluble starch content was higher compared to the soluble starch contents of PSP, LGD and LFD ($P<0.05$; Table 5). The soluble starch content remained stable in LWS during the storage period, whereas in PSP, LGD and LFD increased soluble starch contents were observed (Table 5). At T_{72} there was a significant increase in soluble starch content in LGD and LFD ($P<0.001$), and for PSP the soluble starch content tended to increase ($P<0.10$; Table 5). After 144 hours of storage, the increase in soluble starch was only significant for LFD ($P<0.05$), whereas the increase for PSP and LGD was not significant ($P>0.10$; Table 5).

At T_{72} there was a significant increase in sugar in LWS, LGD and LFD ($P<0.01$; Table 5), and for PSP the sugar content tended to increase ($P<0.10$; Table 5). No significant changes in sugar content in LWS and PSP were observed after 144 hours storage ($P>0.10$), whereas LGD and LFD showed a decrease ($P<0.01$; Table 5) in sugar content. The decline in lactose content in CW was significant at both T_{72} and T_{144} ($P<0.001$; Table 5).

Organic acids and ethanol

At the start of the storage period, the total level of organic acids plus ethanol was higher in LWS compared with PSP, CW, LGD and LFD ($P<0.05$; Table 5). All liquid co-products had a higher initial total level of organic acids and ethanol than the liquid compound diets ($P<0.05$; Table 5).

In PSP, CW, LGD and LFD a significant increase in the total level of organic acids plus ethanol at both T_{72} and at T_{144} was observed, ($P<0.001$; Table 5). In LWS the total level of organic acids plus ethanol tended to increase at T_{72} and at T_{144} ($P<0.10$; Table 5).

Table 5. Initial content, final content and changes during a 6-day storage period in starch, soluble starch, sugar, lactose and total VFA+lactic acid+ethanol content (g/kg) in liquid wheat starch (LWS), mashed potato steam peel (PSP), cheese whey (CW), liquid grower diet (LGD) and liquid finisher diet (LFD)

	PRODUCT					
	LWS	PSP	CW	LGD	LFD	SEM
Starch						
T_0	75.05^{a}	85.26^{b}	-	93.50^{c}	88.27^{bc}	2.014
T_{144}	73.01^{a}	64.27^{b}	-	67.50^{ab}	61.77^{b}	2.597
$T_0 - T_{72}$	1.76	16.49***	-	19.20***	24.90***	
$T_0 - T_{144}$	2.04	21.00***	-	26.00***	26.50***	
Soluble starch						
T_0	20.98^{a}	0.99^{b}	-	2.73^{b}	1.77^{b}	1.145
T_{144}	20.10^{a}	2.82^{b}	-	4.06^{b}	5.92^{b}	1.853
$T_0 - T_{72}$	0.12	-2.02$^{\#}$	-	-5.33***	-6.35***	
$T_0 - T_{144}$	0.88	-1.83	-	-1.33	-4.15*	
Sugar						
T_0	28.99^{a}	1.28^{b}	-	17.94^{c}	21.82^{c}	1.415
T_{144}	24.41^{a}	3.23^{b}	-	8.13^{b}	10.69^{b}	2.790
$T_0 - T_{72}$	3.28**	-1.91$^{\#}$	-	4.50***	-3.10**	
$T_0 - T_{144}$	4.58	-1.96	-	9.81**	11.14**	
Lactose						
T_0	-	-	48.03	-	-	1.725
T_{144}	-	-	35.06	-	-	1.934
$T_0 - T_{72}$	-	-	8.57***	-	-	
$T_0 - T_{144}$	-	-	12.97***	-	-	
Organic acids[†]+ethanol						
T_0	27.92^{a}	7.62^{b}	7.97^{b}	1.59^{c}	1.81^{c}	1.631
T_{144}	31.24^{a}	22.15^{b}	17.08^{b}	40.41^{c}	36.15^{ac}	1.958
$T_0 - T_{72}$	-1.42$^{\#}$	-11.48***	-5.68***	-23.88***	-20.63***	
$T_0 - T_{144}$	-3.32$^{\#}$	-14.53***	-9.11***	-38.81***	-34.34***	

† Lactic acid, formic acid, acetic acid, propionic acid, (iso)butyric acid, (iso)valeric acid (data adapted from Scholten et al., 2001).

a,b,c,d Mean values at time 0 (T_0) or time 144 (T_{144}) within a row with a different superscript are different: P<0.05.

*, **, *** Mean of the time changes differs from zero: # P<0.10; * P<0.05; ** P<0.01; *** P<0.001.

Weight

During storage, the total weight of CW increased, whereas the total weight of LFD decreased ($P<0.01$; Table 6). No changes in total weight of LWS, PSP and LGD were observed ($P>0.10$).

Table 6. Initial weight and changes during a 6-day storage period in total weight, total weight corrected dry matter and total weight moisture in liquid wheat starch (LWS), mashed potato steam peel (PSP), cheese whey (CW), liquid grower diet (LGD) and liquid finisher diet (LFD).

	PRODUCT					
	LWS	PSP	CW	LGD	LFD	SEM
Total weight (kg)						
T_0	45.0	45.0	45.0	45.0	45.0	0.00
$T_0 - T_{144}$	0.00	-0.39	-2.18**	0.17	2.08**	
Total weight of corrected dry matter (kg)						
T_0	10.46^a	6.36^b	3.18^c	11.25^d	11.43^d	0.161
$T_0 - T_{144}$	0.20	0.35*	0.17	0.57**	0.98***	
Total weight of moisture (kg)						
T_0	34.54^a	38.64^b	41.82^c	33.75^d	33.57^d	0.640
$T_0 - T_{144}$	-0.20	-0.74	-2.35**	-0.40	1.10	

^{a,b,c,d} Mean values at time 0 (T_0) within a row with a different superscript are different: $P<0.05$.
*, **, *** Mean of the time changes differs from zero: * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

All liquid feeds showed a numerical loss of total weight of cDM during storage, and the loss was significant for PSP, LGD and LFD ($P<0.05$; $P<0.01$; $P<0.001$, respectively; Table 6). The weight loss was 1.9, 5.5, 5.4, 5.1 and 8.6% of the initial content for cDM of LWS, PSP, CW, LGD and LFD, respectively.

The total weight of moisture in CW increased during the storage period ($P<0.01$; Table 6). LWS, PSP and LGD also showed an increase in moisture concentration during storage, although this was not significant ($P>0.10$; Table 6).

DISCUSSION

Dry matter and corrected dry matter

The DM content of liquid feedstuffs is an important value in optimising the pig diet for a number of reasons (Rijnen and Scholten, 1998). The nutrient contents, expressed in g/kg DM, are used in the calculations for composing liquid diets for pigs. Therefore, the content of nutrients in liquid co-products and diets are analysed, and expressed in g/kg DM. Finally, the liquid feeding system (i.e., the computer) prepares liquid diets based on the DM content of the individual components (Rijnen and Scholten, 1998). In practice, oven drying at 103°C is in general the standard method for determining the DM content of liquid diets and liquid co-products. However, it should be noted that VFA and alcohol become (partly) volatile during oven drying at 103 °C. Therefore, it is advisable to correct the DM content for the volatility of VFA, lactic acid and alcohol (CVB, 1999). In particular, when fermented products with high levels of VFA, lactic acid and alcohol are used, this correction is essential. The values of volatility of lactic acid (8%), VFA's (50%) and alcohol (100%) during oven drying at 103°C, used by CVB (1999), are based on limited research results (CVB, 1999). Additional information about the interaction of temperature, duration of drying and the pH of the product, on the percentage of volatility, is needed to estimate the real DM content of diets containing fermentation products and to optimise the feeding of fermented liquid diets to pigs.

In this study, the initial cDM contents in LWS, PSP, CW, LGD and LFD, expressed as percentage of DM content, were 103.4, 101.0, 101.7, 100.2 and 100.2%, respectively (Tables 3a+b). At the end of the 6-day storage period these percentages had increased to 104.0, 102.6, 104.4, 104.4 and 106.1% for LWS, PSP, CW, LGD and LFD, respectively (Tables 3a+b). This means that the DM content is underestimated, and that this underestimiation becomes larger during the storage period. The results for LWS and PSP agree with the results of Smits (1998), who reported that after a 3-week storage period the cDM was 103.9 and 102.4% of the DM content for LWS and PSP, respectively. Smits (1998) used LWS and PSP originating from the same production plants as used in this trial. Except for the current study there is no additional information on changes of DM and cDM for the products CW, LGD and LFD during storage.

Calculations based on the data in the Tables 3a+b, showed that in LWS, PSP and CW the decline of DM and cDM in the first 72 hours of storage was more rapid compared with that in LGD and LFD. The ratio of the decline in DM at T_{72} expressed to the value at T_{144} was 0.71, 0.60 and 0.66, whereas for LGD and LFD this ratio was 0.30 and 0.36 (Tables 3a+b). For cDM these ratios were 0.77, 0.58, 0.66, 0.09 and 0.43 for LWS, PSP, CW, LGD and LFD, respectively. In addition, it is clear that during the last phase of the storage period (T_{96} to T_{144}), the reduction of

both DM and cDM were remarkably smaller in LWS, PSP and CW compared to LGD and LFD. Therefore it seems that the liquid compound diets were not in a steady state at the end of the 6-day storage.

This study shows changes in total amount of cDM (kg) and the total amount (kg) of moisture in the products during a 6-day storage period. In all products total amount of cDM numerically decreased, whereas the total amount of moisture numerically increased in all products, except LGD (Table 6).

Crude fat, crude protein and true protein

In this study, a minor change in the content of CFAT was observed (Table 7), which may be explained by CFAT being a minor nutrient for microbial growth and activity during fermentation.

The total amounts of CP and TP at the end of storage, as a ratio of the initial amount, varied between 0.96 and 1.06 for CP and 0.95 and 1.05 for TP (Table 7). This indicates that during the 6-day storage period no substantial breakdown of protein occurred. However, it cannot be excluded that there was no limited transformation of protein, due to the proliferation and maintenance of the large microbial population during the six days of storage. Consequently, a small fraction of feed protein is probably transformed into microbial protein, which has a different amino acid composition compared with feed protein. Fermentation and enzymatic digestion of protein and amino acids by microorganisms (e.g yeasts, Coliforms) is undesirable, due to the loss of feeding value and the production of ammonia and toxic amines, that are harmful to the gastrointestinal mucosa (Visek, 1972; 1978). According to Pedersen (1999) about 25% of the added synthetic lysine in liquid weaner diets disappears during 24-hours fermentation. In scientific literature, information regarding the effects of storage of liquid pig diets on the microbial population, protein level, amino acid composition and levels of ammonia and toxic amines is scarce.

Table 7. The total amount of nutrients at T_0 (g per 45 kg product) and at T_{144}, as ratio of the initial amount[1], in liquid wheat starch (LWS), mashed potato steam peel (PSP), cheese whey (CW), liquid grower diet (LGD) and liquid finisher diet (LFD)

	PRODUCT				
	LWS	PSP	CW	LGD	LFD
Crude fat					
T_0 (g)	339.3	63.0	59.9	543.2	793.4
T_{144} [1] (ratio)	0.91	1.11	0.97	0.90	1.01
Crude protein					
T_0 (g)	1411.2	818.6	465.3	2360.7	2180.7
T_{144} [1] (ratio)	1.00	1.00	1.06	1.00	0.96
True protein					
T_0 (g)	761.4	587.3	275.9	2033.1	1857.2
T_{144} [1] (ratio)	1.00	1.05	0.98	0.97	0.95
Starch					
T_0 (g)	3377.3	3836.7	-	4207.5	3972.2
T_{144} [1] (ratio)	0.97	0.76	-	0.72	0.67
Soluble starch					
T_0 (g)	944.1	44.6	-	122.9	79.7
T_{144} [1] (ratio)	0.96	2.87	-	1.48	3.19
Sugar / lactose					
T_0 (g)	1304.6	57.6	2161.4	807.3	981.9
T_{144} [1] (ratio)	0.84	2.55	0.77	0.45	0.47
Organic acids[2] + ethanol					
T_0 (g)	1256.4	342.9	358.7	71.6	81.5
T_{144} [1] (ratio)	1.12	2.93	2.25	25.30	19.04
Gross energy[3]					
T_{144} (ratio)	0.97	0.97	0.99	1.01	0.99

[1] Calculated as: ((amount in kg)$_{T144}$ * (content in g/kg)$_{T144}$) / ((amount in kg)$_{T0}$ * (content in g/kg)$_{T0}$).
[2] Calculated as: Lactic acid + formic acid + acetic acid + propionic acid + (iso)butyric acid + (iso)valerianic acid. Individual data adapted from Scholten et al. (2001).
[3] The ratio is calculated as: (Gross Energy T_{144} / Gross Energy T_0).

Carbohydrates

In all products changes in the carbohydrate composition occurred during storage. However, the changes in LWS were smaller than in the other products (Table 5). This may be due to the fact that the initial pH of LWS was already 3.6 (Scholten et al., 2001), which is in line with the pH found by Smits (1998) and Scholten et al. (1999b). This low pH may be due to the fact that LWS

is fermented quickly, like wheat which has a pH-drop from 5.5 to below 4.0 within 24 hours (Scholten, unpublished data). Furthermore, in the Netherlands it is common practice to add organic acids to LWS to avoid the proliferation of yeasts. This can be done by adding about 2 kg of a mixture of propionic and formic acid per tonne of LWS (Scholten, unpublished data). Smits (1998) reported that at the moment of delivery, the pH of fresh LWS was about 3.4 to 3.8, with lactic and acetic acid present in high concentrations.

In all five tested liquid products, there is a process of transforming carbohydrates into organic acids and ethanol. As can be calculated from Table 5, the total amount of carbohydrates (starch, soluble starch, sugar, lactose) at the end of the storage period, decreased to 0.94, 0.80, 0.73, 0.70 and 0.70% of the initial content for LWS, PSP, CW, LGD and LFD, respectively. The reductions in total carbohydrates in PSP and LWS are in agreement with values reported by Edwards et al. (1986) and Smits (1998). It appears that during fermentation there is a continious breakdown of larger starch molecules into soluble starch and sugars, and subsequently into organic acids and ethanol. Microorganisms like lactic acid bacteria, yeasts and Coliforms are naturally present in pig diets (Geary et al., 1996; Russell et al., 1996; Jensen and Mikkelsen, 1998) and are involved in the transformation of carbohydrates into fermentation products. Yeasts produce mainly CO_2 from glucose and, without the presence of oxygen, also ethanol (Prescott et al., 1996). Homofermentative lactic acid bacteria produce 90% lactic acid from glucose, whereas heterofermentative lactic acid bacteria produce out of glucose 50% lactic acid and 50% acetic acid, ethanol and CO_2 (Prescott et al., 1996). The production of CO_2 means a loss of energy value of the pig diet. The addition of lactic acid to pig diets has improved feed intake substantially, whereas the addition of acetic acid has not had a positive effect on feed intake (reviewed by Scholten et al., 1999b). Therefore, it can be hypothesized that the microbial population may influence the energy value of the diet as well as the performance of the pigs. So far, information on these aspects is very limited. Therefore, research is needed to obtain knowledge about how to prepair and control the chemical and microbial composition of liquid diets to receive the optimal performance of the pigs.

Total amount of nutrients and energy value

Fermentation changed the type of energy supplying nutrients from carbohydrates into lactic acid, VFA and alcohol. After a storage period of six days these nutrients, expressed as a ratio of the initial value, have changed dramatically (Table 7). Based on the results of our experiment, the gross energy content at the start and at the end of the fermentation was calculated. This calculation was based on the specific heat of combustion values of all nutrients. We observed minor changes in gross energy content, expressed as a ratio of the initial value: LWS, PSP, CW,

LGD and LFD had ratios of 0.97, 0.97, 0.99, 1.01 and 0.99, respectively (Table 7). This small reduction is in agreement with results obtained by Jensen and Mikkelsen (1998), who reported a reduction of 0.03 in gross energy content of fermented liquid diets. The small reduction can be the consequence of the transformation from glucose to mainly lactic acid, accompanied by a minor energy loss (Prescott et al., 1996).

ACKNOWLEDGEMENTS

The authors thank Marianne van 't End and Dick Bongers for their assistance with the chemical analyses, and Erik Bruininx and Colm Moran for critically reviewing the manuscript.

REFERENCES

CVB, 1999. Centraal Veevoederbureau. Veevoedertabel. Lelystad, The Netherlands.

Edwards, S.A., Fairbaim, C.B., Capper, A.L., 1986. Liquid potato feed for finishing pigs: feeding value, inclusion rate and storage properties. Anim. Feed Sci. Technol. 15, 129-139.

Geary, T.M., Brooks, P.H., Morgan, D.T., Campbell, A., Russell, P.J., 1996. Performance of weaner pigs fed ad libitum with liquid feed at different dry matter concentrations. J. Sci. Food Agric. 72, 17-24.

Goelema, J.O., Spreeuwenberg, M.A.M., Hof, G., van der Poel, A.F.B., Tamminga, S., 1998. Effect of pressure toasting on the rumen degradability and intestinal digestibility of whole and broken peas, lupins and faba beans and a mixture of these feedstuffs. Anim. Feed Sci. Techn. 76, 35-50.

ISO, 1978. Animal feeding stuffs – Determination of crude ash (ISO 5984). International Organization for Standardization, Genève, Switserland.

ISO, 1979. Animal feeding stuffs – Determination of nitrogen and calculation of crude protein content (ISO 5983). International Organization for Standardization, Genève, Switserland.

ISO, 1983. Animal feeding stuffs – Determination of moisture content (ISO 6496). International Organization for Standardization, Genève, Switserland.

ISO, 1996. Animal feeding stuffs – Determination of fat content, category B (ISO/DIS 6492). International Organization for Standardization, Genève, Switserland.

Jensen, B.B., Mikkelsen, L.L., 1998: Feeding liquid diets to pigs. In: Garnsworthy, P.C., Wiseman, J. (Eds.), Recent Advances in Animal Nutrition. Nottingham University Press, Loughborough, 107-126.

Pedersen, A. Ø., 1999. Styret fermentering af vådfoder. Kongres for Svineproducenter, 26-27 Oktober.

Prescott, L.M., Harley, J.P., Klein, D.A., 1996. Microbiology. WCB Publishers, London. 935 p.

Rijnen, M.M.J.A., Scholten, R.H.J., 1998: Fermentation of liquid diets and by-products during storage. Report P1.211. Praktijkonderzoek Varkenshouderij, Rosmalen, The Netherlands (in Dutch, with English summary).

Russell, P.J., Geary, T.M., Brooks, P.H., Campbell, A., 1996. Performance, water use and effluent output of weaner pigs fed ad libitum with either dry pellets or liquid feed and the role of microbial activity in the liquid feed. J. Sci. Food Agric.73, 8-16.

SAS, 1994. SAS/STAT® User's Guide (Release 6.12 Ed.). SAS Inst. Inc., Cary, NC.

Scholten, R.H.J., Peet-Schwering, C.M.C. van der, Hartog, L.A. den, Vesseur, P.C., Verstegen, M.W.A., 1999a. Effect of liquid by-products on performance and health of pigs. Pig News Info. 20, 81N-88N.

Scholten, R.H.J., Peet-Schwering, C.M.C. van der, Verstegen, M.W.A., Hartog, L.A. den, Schrama, J.W., Vesseur, P.C., 1999b. Fermented co-products and fermented compound diets for pigs: a review. Anim. Feed Sci. Technol. 82, 1-19.

Scholten, R.H.J., Rijnen, M.M.J.A., Schrama, J.W., Boer, H., van der Peet-Schwering, C.M.C., den Hartog, L.A., Vesseur, P.C., Verstegen, M.W.A., 2001. Fermentation of liquid coproducts and liquid compound diets: Part 2. Effects on pH, acid binding capacity, organic acids and ethanol during a 6-day storage period. J. Anim. Physiol. Anim. Nutr. 85, 124-134.

Smits, B., Gelder, A.H. van, Jongbloed, R., Cone, J.W., 1994: Samenstelling van de koolhydraatfractie in aardappelpersvezels, aardappelstoomschillen, Bondatar, mycelium-spoeling, restgist en tarwe-indampconcentraat; een oriënterende studie. Intern rapport ID-DLO no. 417. Instituut voor Dierhouderij en Diergezondheid. Lelystad, The Netherlands (in Dutch).

Smits, B, 1998: Effect van bewaring en droge stofbepaling op de chemische samenstelling en op de mate van vervluchtiging van vluchtige bestanddelen in vochtrijke enkelvoudige diervoeders. Rapport ID-DLO no. 98.031. Instituut voor Dierhouderij en Diergezondheid. Lelystad, The Netherlands (in Dutch).

Stutzer, Barnstein, F., 1900. Landwirtschaftlichesversuchsstat 54, 327.

Visek, W.J., 1972. Effects of urea hydrolysis on cell life-span and metabolism. Fed. Proc. Fed. Am. Soc. Exp. Biol. 31, 1178-1193.

Visek, W.J., 1978. The mode of growth promotion by antibiotics. J. Anim. Sci. 46, 1447-1469.

Weast, R.C., Handbook of Chemistry and Physics, 49th Ed., 1968-1969, The Chemical Rubber Company, Cleveland, Ohio, USA.

Chapter 3

FERMENTATION OF LIQUID CO-PRODUCTS AND LIQUID COMPOUND DIETS: PART 2. EFFECTS ON PH, ACID-BINDING CAPACITY, ORGANIC ACIDS AND ETHANOL DURING A 6-DAY STORAGE PERIOD

Ronald H.J. Scholten[1,*], Martin M.J.A. Rijnen[2], Johan W. Schrama[3], Huug Boer[2], Carola M.C. van der Peet-Schwering[1], Leo A. den Hartog[1], Peter C. Vesseur[1], Martin W.A. Verstegen[2]

[1] Research Institute for Animal Husbandry, Lelystad, The Netherlands

[2] Wageningen Institute of Animal Sciences, Animal Nutrition Group, Wageningen, The Netherlands

[3] Wageningen Institute of Animal Sciences, Fish Culture and Fisheries Group, Wageningen, The Netherlands

[*] Present address: Beuker Vochtrijke Diervoeders B.V., Doetinchem, The Netherlands

Published in:

Reprinted from Journal of Animal Physiology and Animal Nutrition 85 (2001) 124-134

with permission of Blackwell Wissenschafts-Verlag

ABSTRACT

The effects of a 6-day storage period on changes in pH, acid binding capacity, level of organic acids and ethanol of three liquid co-products [liquid wheat starch (**LWS**), mashed potato steam peel (**PSP**) and cheese whey (**CW**)] and two liquid compound diets [liquid grower diet (**LGD**) and liquid finisher diet (**LFD**)] were studied. All products, except LWS, showed a significant decrease in pH and acid binding capacity during storage. At the end of the storage period, all products reached a pH of between 3.5 and 3.9. In general, it can be concluded that the lactic acid content, and to a lesser extent acetic acid content, increased dramatically during storage. In contrast, the ethanol content increased significantly in the liquid compound diets only. The pattern of changes in pH and organic acids during the 6-day storage period was different between the liquid co-products and the liquid compound diets. At the start of storage, liquid co-products are already in the "middle" of the fermentation process, while liquid compound diets need approximately 24 to 36 hours before fermentation begins. Consequently, in practice a different approach to obtain fermented diets is needed for liquid co-products and liquid compound diets.

INTRODUCTION

Feeding fermented liquid diets to pigs results in improved growth performance, as has been observed when pigs were fed fermented diets compared with non-fermented diets (Russell et al., 1996; Jensen and Mikkelsen, 1998; Scholten et al., 1999). Analyses of the fermented pig diets at the moment they were fed showed that these diets were characterized by high numbers of microbial populations, a low pH (3.5 to 4.5) and high concentrations of microbial end metabolites (i.e., lactic acid, volatile fatty acids and alcohol) (Scholten et al., 1999). These characteristics may contribute to the improved growth performance. Several authors have reported that a reduction in pH and acid binding capacity of the diet and the addition of organic acids to the diet improved the daily gain, feed intake and feed conversion ratio of pigs (review by Partanen and Mroz, 1999). Based on this information, fermentation of diets may be a method to enhance growth performance of pigs. Furthermore, the use of fermented diets may also be an alternative for the prophylactic use of antimicrobial growth promoters in pig diets (Scholten et al., 1999).

Until now, little has been known about the kinetics of the fermentation process of liquid co-products and liquid compound diets. In general, liquid wheat starch (LWS), mashed potato steam peel (PSP) and cheese whey (CW) are the most commonly used liquid co-products in

Europe and, in practice, they are fed mainly to growing-finishing pigs (Scholten et al., 1999). Therefore, the aim of the present study was to examine the changes in pH, acid binding capacity, organic acid and ethanol contents in LWS, PSP, CW, a liquid grower diet (LGD) and a liquid finisher diet (LFD) during a 6-day storage period.

MATERIALS AND METHODS

Products and storage

The initial chemical composition of LGD and LFD is given in Table 1. The initial chemical composition of LWS, PSP and CW is published elsewhere (Scholten et al., 2001).

Table 1. Composition (g/kg) of the grower feed and finisher feed used for the liquid grower diet (LGD) and the liquid finisher diet (LFD)

	LGD	LFD
Wheat	125.0	34.0
Barley	175.0	-
Peas	68.0	123.0
Rape seed, extracted	81.0	175.0
Soya beans, extracted	160.0	88.0
Sunflower seed, extracted	50.0	21.0
Wheat middlings	36.0	48.0
Maize glutenfeed	-	27.0
Molasses, beet	20.0	30.0
Tapioca	233.5	400.0
Fat	24.5	42.0
Limestone	10.0	3.3
Salt	2.0	1.7
Rukanaphos	5.7	1.8
Premix	5.0[1]	2.5[2]
Phytase	2.3	2.4
Methionine-50%	0.6	0.3
Lysine-40%	1.4	-

[1] No acids; 50 mg olaquindox; 160 mg Cu; 8000 IE Vit A; 1500 IE Vit D3; 30 IE Vit E.
[2] No acids; 45 mg zincbacitrine; 25 mg Cu; 6000 IE Vit A; 1200 IE Vit D3; 25 IE Vit E.

The experiment consisted of a storage period of six days (144 hours), which was repeated three times. Approximately one hour before the start of each storage period, fresh batches of LWS, PSP and CW were delivered from the production plants. The grower and finisher diets were both pelleted compound diets, each from one batch from a compound feed factory. One hour before the start of each storage period, the grower and finisher diets were mixed with water in a water to feed ratio of 2.5:1. Both LGD and LFD were soaked and stirred continuously for one hour, before starting the storage period (T_0).

The storage of the products and the method of sampling are described by Scholten et al. (2001). All products were stirred automatically for one minute every two hours. PSP was not stirred because no separation was expected due to its high viscosity and because, in practice, this product is also not stirred. Samples (500 ml) were taken at 0, 12, 24, 36, 48, 72, 96 and 144 hours after the start of each storage period (T_0, T_{12}, T_{24}, T_{36}, T_{48}, T_{72}, T_{96} and T_{144}, respectively). Directly before sampling, the tanks with LWS, CW, LGD and LFD were additionally stirred for one minute. All samples were immediately stored at -20°C.

Chemical analyses

All samples were analysed for pH, formic acid, acetic acid, propionic acid, (iso)butyric acid, (iso)valeric acid, lactic acid and ethanol. The acid binding capacity was analysed in two samples (T_0 and T_{144}).

The pH was measured using a Schott-CG841 pH analyser (Schott, Hofheim, Germany). Acid binding capacity was measured according to Prohászka and Baron (1980); 20.0 ml 0.1 N HCl was added to 2.0 g DM of the product, followed by titration with NaOH to reach pH 3.0.

Samples for the determination of organic acids (C2-C5) were centrifuged (41.750 G; 4°C) and the supernatant was extracted from the sample and diluted with phosphoric acid in a ratio of 20:1. Organic acids (C2-C5) were analysed by Gas Chromatography (**GC**) (Williams et al., 2000). Samples for the determination of lactic acid (C6) and formic acid (C1) were centrifuged (41.750 G; 4°C) and the supernatant was extracted from the sample and subsequently acidified to pH 2.0 by the addition of a small volume of sulphuric acid. Lactic acid, formic acid and ethanol were quantified by High Performance Liquid Chromatography (**HPLC**) (Ehrlich et al., 1981).

Statistical analyses

All statistical procedures were carried out using the General Linear Model procedure of SAS (SAS, 1994). Preliminary analyses showed no differences among replicates. Therefore,

'replicates' was not used as factor in the model. The used model was:

$$Y_{ij} = \mu + P_i + e_{ij}$$

where Y_{ij} = dependent variable; μ = overall mean; P_i = fixed effect of product (i = 1, .., 5); e_{ij} = error term. The results of the model were used to determine if the change in time was different from zero (probability > |T| H0: LSMEAN = 0; SAS, 1994).

RESULTS

pH

At the start of the storage period (T_0), the pH differed between the liquid co-products LWS, PSP and CW (3.61, 4.40, 4.10, respectively; P<0.05; Table 2). At the end of the 6-day storage period, the pH of LWS, PSP and CW did not differ, and was on average 3.50 (P>0.10; Table 2).

Table 2. Initial pH, final pH and changes in pH of liquid wheat starch (LWS), mashed potato steam peel (PSP), cheese whey (CW), liquid grower diet (LGD) and liquid finisher diet (LFD) during a 6-day storage period

	PRODUCT					
	LWS	PSP	CW	LGD	LFD	SEM
T_0	3.61^a	4.40^b	4.10^c	5.74^d	5.61^d	0.077
T_{144}	3.51^a	3.53^a	3.46^a	3.77^b	3.89^c	0.031
$T_0 - T_{12}$	0.03	0.43***	0.10*	-0.07$^{\#}$	-0.04	
$T_0 - T_{24}$	0.04	0.61***	0.21***	-0.06	-0.04	
$T_0 - T_{36}$	0.05	0.70**	0.32$^{\#}$	0.38*	0.57**	
$T_0 - T_{48}$	0.07	0.70***	0.38***	1.02***	1.22***	
$T_0 - T_{72}$	0.05	0.72***	0.51***	1.49***	1.47***	
$T_0 - T_{96}$	0.08	0.77***	0.58***	1.80***	1.59***	
$T_0 - T_{144}$	0.10	0.87***	0.64***	1.97***	1.72***	

^{a,b,c} Mean values at time 0 (T_0) or time 144 (T_{144}) within a row with a different superscript are different: P<.05.

*, **, *** Mean of the time changes differs from zero; $^{\#}$ P<.10; * P<.05; ** P<.01; *** P<.001.

At both T_0 and T_{144} the pH of the liquid co-products was lower than the pH of the liquid diets (P<0.05; Table 2). At T_0, the pH of LGD and LFD did not differ (P>0.10; Table 2), whereas at T_{144} the pH of LGD was lower than that of LFD (3.77 and 3.89, respectively, P<0.05; Table 2).

The pH of LWS was stable during the whole 6-day storage period (Table 2), whereas the pH of PSP and CW decreased during storage. The pH of PSP and CW showed at rapid decline immediately after the start of the storage period. At T_{12}, the pH of PSP and CW was lower than at T_0 ($P<0.001$ and $P<0.05$, respectively; Table 2). In contrast, the pH of LGD and LFD increased numerically during the first 24 hours of storage, followed by a rapid decline. At T_{36} the pH of LGD and LFD was lower compared with T_0 ($P<0.05$ and $P<0.01$, respectively; Table 2).

Acid Binding Capacity

At T_0, the acid binding capacity of LGD and LFD was higher than that of LWS, PSP and CW ($P<0.05$; Table 3). CW had the lowest acid binding capacity ($P<0.05$; Table 3).

At T_{144}, the acid binding capacity of LGD and LFD was still higher compared with LWS, PSP and CW ($P<0.05$; Table 3). The acid binding capacity of CW remained the lowest ($P<0.05$; Table 3). With the exception of LWS, the acid binding capacity of all products decreased during the 6-day storage ($P<0.001$; Table 3).

Table 3. Initial, final and changes in acid binding capacity (meq 0.1 N HCL/ kg) in liquid wheat starch (LWS), mashed potato steam peel (PSP), cheese whey (CW), liquid grower diet (LGD) and liquid finisher diet (LFD) during a 6-day storage period

	PRODUCT					
	LWS	PSP	CW	LGD	LFD	SEM
T_0	77.0^a	79.5^a	60.0^b	199.5^c	169.2^d	3.99
T_{144}	74.5^a	64.2^a	48.8^b	161.2^c	156.6^c	3.78
$T_0 - T_{144}$	2.5	15.3***	11.2***	38.3***	12.6***	

^{a,b,c} Mean values at time 0 (T_0) or time 144 (T_{144}) within a row with a different superscript are different: $P<.05$.

*, **, *** Mean of the time changes differs from zero; * $P<.05$; ** $P<.01$; *** $P<.001$.

Lactic acid

At T_0, the lactic acid content of the liquid co-products was higher than in the liquid diets ($P<0.05$; Table 4). However, after a 6-day storage period the lactic acid contents of LGD and LFD were higher compared to LWS, PSP and CW ($P<0.05$; Table 4). Despite the similar lactic acid content at T_0, the lactic acid content of LGD was higher than that of LFD at the end of the storage period ($P<0.05$; Table 4).

Table 4. Initial content, final content and changes in lactic acid (g/kg) in liquid wheat starch (LWS), mashed potato steam peel (PSP), cheese whey (CW), liquid grower diet (LGD) and liquid finisher diet (LFD) during a 6-day storage period

	PRODUCT					
	LWS	PSP	CW	LGD	LFD	SEM
T_0	15.81^{a}	5.80^{b}	6.78^{b}	0.47^{c}	0.61^{c}	1.015
T_{144}	17.04^{ab}	18.67^{a}	14.87^{b}	30.76^{c}	22.41^{d}	1.052
$T_0 - T_{12}$	0.01	-5.18***	-0.82*	0.07	-0.03	
$T_0 - T_{24}$	-0.17	-7.87***	-1.58**	0.02	-0.07	
$T_0 - T_{36}$	-0.87	-9.00***	-2.70	-4.26*	-5.54**	
$T_0 - T_{48}$	-0.50	-9.99***	-3.72**	-11.92***	-13.04***	
$T_0 - T_{72}$	-0.65	-10.34***	-5.04***	-18.92***	-17.49***	
$T_0 - T_{96}$	-0.73	-11.41***	-6.35***	-25.37***	-19.09***	
$T_0 - T_{144}$	-1.23	-12.87***	-8.09***	-30.28***	-21.80***	

^{a,b,c} Mean values at time 0 (T_0) or time 144 (T_{144}) within a row with a different superscript are different: P<.05.

*, **, *** Mean of the time changes differs from zero; * P<.05; ** P<.01; *** P<.001.

With the exception of LWS, the lactic acid content of all products increased during the 6-day storage period (P<0.001). However, the rate of this increase was different between the liquid co-products (i.e., PSP and CW) and the liquid diets. PSP and CW already showed an increase in lactic acid content at T_{12} (P<0.001 and P<0.05, respectively), whereas LGD and LFD showed no time effects on lactic acid until T_{36} (P<0.05 and P<0.01, respectively; Table 4).

Acetic acid

At both T_0 and T_{144} the acetic acid content of LWS was higher than those of the other products (P<0.05; Table 5). Except for the difference between CW and LGD at T_{144}, there were no differences in acetic acid contents between PSP, CW, LGD and LFD at T_0 and T_{144} (P>0.10; Table 5).

The acetic acid content of all products increased during the 6-day storage period (P<0.05), with the exception of the acetic acid content of CW. However, the rate of this increase was different between the liquid co-products (i.e., LWS and PSP) and the liquid diets. LWS and PSP showed an increase in acetic acid content by T_{12}, whereas LGD and LFD showed no time effects on acetic acid until T_{36} and T_{48}, respectively (Table 5).

Table 5. Initial content, final content and changes in acetic acid (g/kg) in liquid wheat starch (LWS), mashed potato steam peel (PSP), cheese whey (CW), liquid grower diet (LGD) and liquid finisher diet (LFD) during a 6-day storage period

	PRODUCT					
	LWS	PSP	CW	LGD	LFD	SEM
T_0	5.00^{a}	0.94^{b}	0.30^{b}	0.14^{b}	0.18^{b}	0.736
T_{144}	6.75^{a}	2.18^{bc}	0.52^{c}	2.63^{b}	1.84^{bc}	0.653
$T_0 - T_{12}$	-0.32*	-0.54**	0.05	-0.03	-0.02	
$T_0 - T_{24}$	-0.35$^{\#}$	-0.52*	0.00	-0.03	0.00	
$T_0 - T_{36}$	-0.81*	-0.78*	-0.02	-0.86*	-0.44	
$T_0 - T_{48}$	-0.70*	-0.76*	-0.02	-1.64***	-1.11**	
$T_0 - T_{72}$	-0.75*	-0.92**	-0.06	-2.07***	-1.27***	
$T_0 - T_{96}$	-0.91*	-1.12*	-0.10	-2.37***	-1.31**	
$T_0 - T_{144}$	-1.75**	-1.24*	-0.21	-2.49***	-1.65**	

^{a,b,c} Mean values at time 0 (T_0) or time 144 (T_{144}) within a row with a different superscript are different: P<.05.

*, **, *** Mean of the time changes differs from zero; $^{\#}$ P<.10; * P<.05; ** P<.01; *** P<.001.

Other Acids

At both T_0 and T_{144} the propionic acid content was higher in LWS than in the other products (P<0.05; Table 6). At T_{144}, the propionic acid content of PSP was higher than those of CW, LGD and LFD (P<0.05; Table 6). During storage, no significant changes in the propionic acid content were observed in the examined products (P>0.10; Table 6).

At T_0 and T_{144} the formic acid content of LWS was higher than those of PSP, CW, LGD and LFD (P<0.05; Table 6). During the 6-day storage period, no changes in formic acid content were observed in the liquid co-products (P>0.10), whereas the formic acid content of the liquid diets increased during storage (P<0.001; Table 6).

At both T_0 and T_{144} the valeric acid content of LWS was higher than those of the other products (P<0.05; Table 6). No differences or changes in the isobutyric acid, butyric acid and isovaleric acid contents occurred between the five products during the 6-day storage period (P>0.10; Table 6).

Table 6. Initial content, final content and changes in propionic acid, formic acid, (iso)butyric acid and (iso)valeric acid (g/kg) in liquid wheat starch (LWS), mashed potato steam peel (PSP), cheese whey (CW), liquid grower diet (LGD) and liquid finisher diet (LFD) during a 6-day storage period

	PRODUCT					
	LWS	PSP	CW	LGD	LFD	SEM
Propionic acid						
T_0	1.52^a	0.22^b	0.14^b	0.04^b	0.08^b	0.058
T_{144}	1.57^a	0.20^b	0.07^c	0.07^c	0.07^c	0.039
$T_0 - T_{144}$	-0.05	0.02	0.07	-0.03	0.01	
Formic acid						
T_0	1.77^a	0.45^b	0.45^b	0.45^b	0.37^b	0.064
T_{144}	1.74^a	0.51^b	0.51^b	0.86^c	0.67^{bc}	0.092
$T_0 - T_{144}$	0.03	-0.06	-0.06	-0.41***	-0.30***	
Butyric acid						
T_0	0.21	0.04	0.01	0.00	0.00	0.049
T_{144}	0.41	0.03	0.01	0.00	0.01	0.101
$T_0 - T_{144}$	-0.20	0.01	0.00	0.00	-0.01	
Isobutyric acid						
T_0	0.00	0.00	0.01	0.00	0.00	0.003
T_{144}	0.02	0.00	0.01	0.00	0.00	0.007
$T_0 - T_{144}$	-0.02	0.00	0.00	0.00	0.00	
Valeric acid						
T0	2.97^a	0.12^b	0.16^b	0.48^b	0.53^b	0.426
T144	3.05^a	0.23^b	0.18^b	0.53^b	0.45^b	0.329
$T_0 - T_{144}$	-0.08	-0.11	-0.02	-0.05	0.08	
Isovaleric acid						
T_0	0.06	0.00	0.00	0.00	0.00	0.016
T_{144}	0.19	0.00	0.00	0.10	0.10	0.052
$T_0 - T_{144}$	-0.13	0.00	0.00	-0.10	-0.10	

^{a,b,c} Mean values at time 0 (T_0) or time 144 (T_{144}) within a row with a different superscript are different: P<.05.

*, **, *** Mean of the time changes differs from zero; *** P<.001.

Ethanol

The initial ethanol content of LWS was higher than those of the other products ($P<0.05$; Table 7). At T_{144}, the ethanol contents of LWS, PSP and CW did not differ($P>0.10$; Table 7). At T_{144}, the ethanol content in LFD was higher ($P<0.05$) than the ethanol content of LGD, which was higher ($P<0.05$) than the ethanol contents of the liquid co-products (Table 7).

The ethanol contents of the liquid co-products remained stable during the 6-day storage period. In contrast, the ethanol contents of the liquid diets increased during the storage period ($P<0.001$; Table 7).

Table 7. Initial content, final content and changes in ethanol (g/kg) in liquid wheat starch (LWS), mashed potato steam peel (PSP), cheese whey (CW), liquid grower diet (LGD) and liquid finisher diet (LFD) during a 6-day storage period

	PRODUCT					
	LWS	PSP	CW	LGD	LFD	SEM
T_0	0.60^a	0.04^b	0.12^b	0.00^b	0.00^b	0.068
T_{144}	0.48^a	0.33^a	0.90^a	5.47^b	10.59^c	1.606
$T_0 - T_{12}$	0.04	-0.03	-0.11	0.00	0.00	
$T_0 - T_{24}$	0.08	-0.33***	-0.26***	0.00	0.00	
$T_0 - T_{36}$	0.13	-0.28	-0.27	-0.46	-0.72*	
$T_0 - T_{48}$	0.03	-0.19	-0.45	-1.60***	-1.50***	
$T_0 - T_{72}$	-0.04	-0.13	-0.58#	-2.77***	-1.83***	
$T_0 - T_{96}$	0.07	-0.26	-0.74	-4.29***	-4.54***	
$T_0 - T_{144}$	0.12	-0.29	-0.78	-5.47***	-10.59***	

^{a,b,c} Mean values at time 0 (T_0) or time 144 (T_{144}) within a row with a different superscript are different: $P<.05$.

*, **, *** Mean of the time changes differs from zero; # $P<.10$; * $P<.05$; ** $P<.01$; *** $P<.001$.

DISCUSSION

pH-value

The low pH-value and high concentration of fermentation acids and ethanol at T_0, and subsequent no change in these values, suggest that the fermentation process in LWS was complete before the start of the storage period (Figure 1). One explanation for this might be the rapid fermentation process of wheat. A preliminary study showed that within 24 hours the pH-

value of a mixture of hammer milled wheat with water dropped from 5.5 to below 4.0 (Scholten, unpublished data). A second explanation for the low and stable pH is the addition of 2 kg of a mixture of propionic + formic acid per 1000 kg LWS at the factory, just before delivery of LWS to farmers (Scholten, unpublished). The fermentation pattern of LWS in this experiment is in agreement with the results of Smits (1998), who used LWS of the same origine. Smits (1998) reported a high lactic acid and ethanol content at the start of the storage period and a stable pH of 3.5 for LWS during a storage period of three weeks.

In contrast to LWS, the decline in pH in PSP and CW showed that at T_0 these liquid co-products were actively fermenting (Figure 1), whereas both liquid compound diets were at the start of their fermentation process at T_0. The pH of both liquid compound diets showed a slight increase during the first 24 hours, followed by a rapid decline between T_{36} and T_{72}. A more stable pH was established during the last phase of storage (Figure 1).

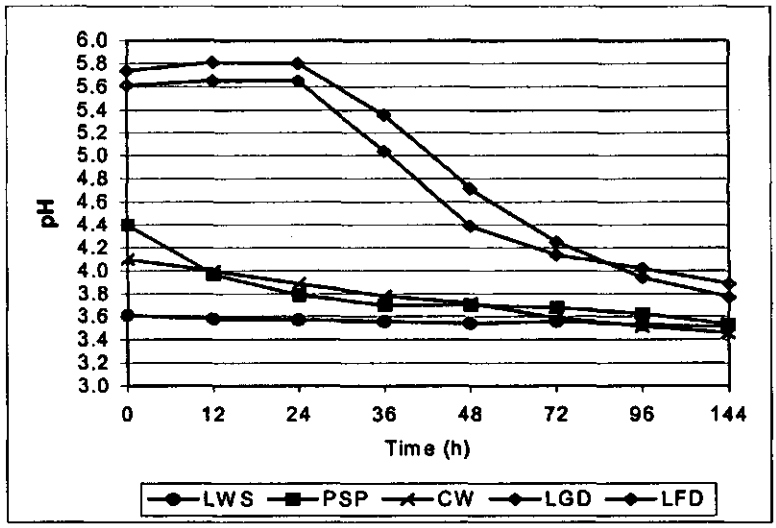

Figure 1. pH pattern during 6-day storage period of liquid wheat starch (LWS), mashed potato steam peel (PSP), cheese whey (CW), liquid grower diet (LGD) and liquid finisher diet (LFD)

During the first 48 hours, the pH-values of both liquid compound diets were above 4.5, which can allows the rapid multiplication of undesirable microbes such as *Salmonella spp* and Coliforms (Nout et al., 1989). In fermented diets, with a pH-value below 4.5, the number of Coliforms is reduced compared with non-fermented diets with a pH above 5 to 5.5 (Russell et al., 1996; Jensen and Mikkelsen, 1998). Feeding fermented diets to weanling pigs caused a

significant reduction in the number of Coliforms throughout the whole gastrointestinal tract (Mikkelsen and Jensen, 1997, 1998). Because Coliforms are associated with the occurrence of post weaning health problems (e.g. diarrhoea) among piglets, fermented diets with a pH below 4.5 may stimulate the health of the weaned pig.

Acid binding capacity

The present study showed that at T_0 the acid binding capacity and the pH of the liquid co-products were lower than those of the liquid compound diets. During the 6-day storage period, the acid binding capacity of the liquid compound diets decreased significantly. However, at T_{144} acid binding capacity of the liquid compound diets was still significantly higher compared with those of the liquid co-products. Several studies have shown that in weanling pigs, diets with a high acid binding capacity increased the number of Coliforms in the stomach (Prohászka and Baron, 1980), produced higher gastric ammonia concentrations (Eidelsburger et al., 1992b), resulted in higher incidence of diarrhoea (Prohászka and Baron, 1980; Eidelsburger et al., 1992b) and had an unfavourable effect on feed conversion rate (Roth and Kirchgessner, 1989). The addition of organic acids to the diets reduced the acid binding capacity (Eidelsburger et al., 1992a). In our experiment, the liquid co-products had a lower acid binding capacity compared with liquid compound diets (Table 3). Further, the acid binding capacity of the LGD was higher compared with that of LFD, which is in contrast to the fact that the younger pig will have more problems acidifying their stomach content (Bolduan et al., 1988). Furthermore, in all products, except LWS, the fermentation process reduced the acid binding capacity, which may be beneficial for the growth performance and health of the pigs.

Fermentation products

The present experiment showed a clear increase in lactic acid contents of the examined products during the 6-day storage period. For LWS, PSP, CW, LGD and LFD the ratio between lactic acid at T_{144} and T_0 was 1.08, 3.22, 2.19, 65.45 and 36.74, respectively (Table 4). Smits (1998) studied the storage of LWS and PSP originating from the same factories as in the current experiment, and reported a ratio of 1.10 and 1.43 for LWS and PSP, respectively, after a storage period of three weeks. The ratio for LWS is similar to that in the present study, whereas the ratio for PSP is lower than that found in the present study. This is probably due to the higher lactic acid content (approximately 11 g/kg) and the lower pH-value (3.8) of PSP at the start of the storage period in the study of Smits (1998) compared with the present study. At the end of the storage, both the study of Smits (1998) and the present study showed a comparable lactic acid content (14.5 g/kg and 18.7 g/kg, respectively) and pH (3.6 and 3.5, respectively).

At the end of the storage period, the level of lactic acid varied between 14.87 and 18.67 g/kg for the liquid co-products, whereas the level of lactic acid in the liquid compound diets varied between 22.41 and 30.76 g/kg. The addition of lactic acid to dry pig compound feed (Roth et al., 1993; Jongbloed and Jongbloed, 1996) or drinking water (Cole et al., 1966), is associated with a favourable effect on growth performance, which might be due to the observed reduction in pH and the number of Coliforms in the stomach (Cole et al., 1966; Thomlinson and Lawrence, 1981; Ratcliffe et al., 1986). Because lactic acid is the dominant organic acid in fermented diets, it can be hypothesized that lactic acid plays an important role in preventing Coliforms in the diet as well in the gastrointestinal tract.

Because the microbial population determines both the type and the proportion of fermentation products produced during the fermentation of carbohydrates, the microbial population may be an explanation for the observed differences in the contents of different fermentation products (Scholten et al., 2001). In the present study, LGD and LFD had different levels and types of fermentation products. After the 6-day storage period, the pH in LFD was significantly higher and the absolute level of lactic acid in LFD was significantly lower than in LGD. In contrast, the production of ethanol was significantly higher in LFD than in LGD. These differences may indicate that the composition of the microbial population differed between both compound diets. So far, however, information on the effects of feed compositions on the microbial population and consequently fermentation patterns of liquid diets and liquid co-products is lacking. It might be possible that the fermentation pattern is affected by differences in feed composition (e.g. raw materials, premixes) and acid binding capacity (e.g. content of limestone, level of crude protein). Additional knowledge is necessary to optimise the fermentation process and to prevent micro-organisms producing undesirable end metabolites through fermentation of carbohydrates or protein into, for example CO_2, ammonia and toxic amines.

The microbial population in the liquid diet may affect the proportions of lactic acid and acetic acid that are produced. In this experiment, for all products, except LWS, the ratio of lactic acid to acetic acid increased during the storage period (Tables 4 and 5). The ratio of lactic acid to acetic acid at the end of the storage period was 2.5, 8.6, 28.6, 11.7 and 12.2 for LWS, PSP, CW, LGD and LFD, respectively. According to Jensen and Mikkelsen (1998) the ratio of lactic acid to acetic acid varied between 5:1 and 10:1 for fermented compound diets. Scholten et al. (1999) observed that there are large differences in the ratio of lactic acid to acetic acid in liquid co-products and liquid compound diets, varying between 3:1 and 30:1. Apart from the fact that microflora influence the type and proportions of fermentation products, they also may influence the performance of the pigs. In human food industry, lactic acid is used as flavour enhancer (Shelef, 1994). In pig diets, lactic acid seems to improve palatability. From a limited number of

experiments, it seems that the addition of lactic acid to pig diets increases feed intake in pigs, whereas the addition of acetic acid does not increase feed intake (reviewed by Scholten et al., 1999). Piglets fed fermented diets had a higher feed intake compared with piglets fed non-fermented diets (Russell et al., 1996; Jensen and Mikkelsen, 1998). Very little is known about the shifts in microbial population and the production of fermentation products during the fermentation process of pig diets, and the effect on feeding value, growth performance and pig health. Specific studies are necessary to elucidate the modes of action of fermented diets, which may possibly lead to the more successful application of fermented diets in pig nutrition and may potentially lead to the development of alternatives for the use of anti-microbial growth enhancers in pig diets.

ACKNOWLEDGEMENTS

The authors thank Berry Diekema and Dick Bongers for their assistance with the chemical analysis, and Erik Bruininx and Colm Moran for critically reviewing the manuscript.

REFERENCES

Bolduan, G., Jung, H., Schneider, R., Block, J., Klenke, B., 1988. Die Wirkung von Propion- und Ameisensäure in der Ferkelaufzucht. J. Anim. Physiol. Anim. Nutr. 59, 72-78.

Cole, D.J.A., Beal., R.M., Luscombe, J.R., 1968. The effect on performance and bacterial flora of lactic acid, propionic acid, calcium propionate and calcium acrylate in the drinking water of weaned pigs. Vet. Rec. 83, 459-464.

Ehrlich, G.G., Goerlitz, D.F., Bourell J.H., Eisen, G.V., Godsy, E.M., 1981. Appl. Environ. Microbiol. 42, 5, 878.

Eidelsburger, U., Roth, F.X., Kirchgessner, M., 1992a. Zum Einfluß von Ameisensäure, Calciuformiat und Natriumhydrogencarbonat auf tägliche Zunahmen, Futteraufnahme, Futterverwertung und Verdaulichkeit. J. Anim. Physiol. Anim. Nutr. 67, 258-267.

Eidelsburger, U., Kirchgessner, M., Roth, F.X., 1992b. Zum Einfluß von Ameisensäure, Calciuformiat und Natriumhydrogencarbonat auf pH-wert, Trockenmassegehalt, konzentration an Carbonsauren und ammoniak in verschiedenen Segmenten des Gastrointestinaltraktes. J. Anim. Physiol. Anim. Nutr. 68, 20-32.

Jensen, B.B., Mikkelsen, L.L., 1998: Feeding liquid diets to pigs. In: Garnsworthy, P.C., Wiseman, J. (Eds.), Recent Advances in Animal Nutrition. Nottingham University Press, Loughborough, 107-126.

Jongbloed, A.W., Jongboed, R., 1996. The effect of organic acids in diets for growing pigs on enhancement of microbial phytase efficacy. Report ID-DLO no. 96009. Lelystad, The Netherlands.

Mikkelsen, L.L., Jensen, B.B., 1997. Effect of fermented liquid feed (FLF) on growth performance and microbial activity in the gastrointestinal tract of weaned piglets. In: Laplace, J.P., Fevrier, C., Barbeau, A. (Eds.), Digestive Physiology in Pigs, E.A.A.P. publication 88, Saint Malo, France, 639-642.

Mikkelsen, L.L., Jensen, B.B., 1998. Performance and microbial activity in the gastrointestinal tract of piglets fed fermented liquid feed at weaning. J. Anim. Feed Sci. 7, 211-215.
Nout, M.J.R., Rombouts, F.M., Havelaar, A., 1989. Effect of accelerated natural lactic fermentation of infant food ingredients on some pathogenic microorganisms. Int. J. Food Microbiol. 8, 351-361.
Partanen, K., Mroz, Z., 1999. Organic acids for performance enhancement in pig diets. Nutr. Res. Rev. 12, 1-30.
Prescott, L.M., Harley, J.P., Klein, D.A., 1996. Microbiology. WCB Publishers, London, 935 p.
Prohászka, L., Baron, F., 1980. The predisposing role of high dietary protein supplies in enteropathogenic E.coli infections of weaned piglets. Zbl. Vet. Med. B. 28, 222-232.
Ratcliffe, B., Cole, C.B., Fuller, R., Newport, M.J., 1986. The effect of yoghurt and milk fermented with a porcine intestinal strain of *Lactobacillus reuteri* on the performance and gastrointestinal flora of pigs weaned at two days of age. Food Microbiol. 3, 203-211.
Roth, F.X., Kirchgessner, M., 1989. Bedeutung von pH-wert und Pufferkapazität des Futters für die Ferkelfütterung – 3. Mitteilung: Effekt einer Erhöhung der Pufferkapazität des Futters durch Natrium- und Kaliumbicarbonat-Zusatz auf Wachstum und Futterverwertung. Landwirtschaft Forschung 42, 176-182.
Roth, F.X., Kirchgessner, M., 1993. Zur Nutritiven Wirksamkeit von Milchsäure in der Ferkelaufzucht. Agribiol. Res. 46, 229-239.
Russell, P.J., Geary, T.M., Brooks, P.H., Campbell, A., 1996. Performance, water use and effluent output of weaner pigs fed ad libitum either dry pellets or liquid feed and the role of microbial activity in the liquid feed. J. Sci. Food Agric.73, 8-16.
SAS, 1994. SAS/STAT® User's Guide (Release 6.12 Ed.). SAS Inst. Inc., Cary, NC.
Scholten, R.H.J., Peet-Schwering, C.M.C. van der, Verstegen, M.W.A., Hartog, L.A. den, Schrama, J.W., Vesseur, P.C., 1999. Fermented co-products and fermented compound diets for pigs: a review. Anim. Feed Sci. Technol. 82, 1-19.
Scholten, R.H.J., Rijnen, M.M.J.A., Schrama, J.W., Boer, H., van der Peet-Schwering, C.M.C., den Hartog, L.A., Vesseur, P.C., Verstegen, M.W.A., 2001. Fermentation of liquid coproducts and liquid compound diets: Part 1. Effects on chemical composition during a 6-day storage period. J. Anim. Physiol. Anim. Nutr. 85, 111-123.
Shelef, L.A., 1994. Antimicrobial effects of lactates: a review. J. Food Protection 57, 445-450.
Smits, B, 1998: Effect van bewaring en droge stofbepaling op de chemische samenstelling en op de mate van vervluchtiging van vluchtige bestanddelen in vochtrijke enkelvoudige diervoeders. Rapport ID-DLO no. 98.031. Instituut voor Dierhouderij en Diergezondheid. Lelystad, The Netherlands (in Dutch).
Thomlinson, J.R., Lawrence, T.L.J., 1981. Dietary manipulations of gastric pH in the prophylaxis of enteric disease in weaned piglets. Vet. Rec. 109, 120-122.
Williams, B.A., Oostdam, A.J., Groot, J.C.J., Boer, H., Tamminga, S., 2000. J. Sci. Food Agric. 80, 484.

Chapter 4

FERMENTATION OF LIQUID WHEAT: EFFECTS OF THE ADDITION OF A STARTER CULTURE AND DIFFERENT PROPORTIONS OF PRE-FERMENTED WHEAT

Colm A. Brooks[1,*], Ronald H.J. Scholten[2,¶], Peter H. Brooks[1], Martin W.A. Verstegen[3]

[1] University of Plymouth, Seale-Hayne Faculty, Devon, England

[2] Research Institute for Animal Husbandry, Lelystad, The Netherlands

[3] Wageningen Institute of Animal Sciences, Animal Nutrition Group,, Wageningen, The Netherlands

[*] Present address: Alltech Biotechnology Inc., Lexington, Kentucky, United States of America

[¶] Present address: Beuker Vochtrijke Diervoeders B.V., Doetinchem, The Netherlands

Submitted to:

Animal Feed Science and Technology

ABSTRACT

The objective of the study was to examine the chemical and microbiological characteristics of fermented, liquid milled wheat (**LMW**). LMW was prepared by hammer-milling dry wheat and mixing with water (1:3 wt/wt) at 30° C. Five fermentation treatments were conducted in 45 litre, closed, PVC containers housed in a room at 24 ± 1 °C, and stored for 48 hours. The Control treatment consisted of LMW with no additions. Four further treatments investigated the benefits of inoculating the LMW or the addition of previously fermented LMW (back-slopping). The 0% backslop treatment (**0% BS**) consisted of 20 kg LMW with about 210 g DM/kg, inoculated with 10 ml each of an overnight culture of *Lactobacillus plantarum* and *Pediococcus pentosaceus* (Alltech Inc., Kentucky, USA). In the three other treatments pre-fermented LMW, prepared as above, was added to freshly prepared LMW so that the pre-fermented wheat comprised 20, 33 or 42% of the final fermentation mixture (**20% BS, 33% BS, 42% BS** respectively). Lactic acid bacteria (**LAB**) counts increased rapidly over the 48-hour steeping period and did not differ significantly between the control and inoculated fermentations at the 24 and 48 h sampling times. By 48 h the pH of all treatments was 3.8 or less. In the back-slopping treatments, coliform bacteria counts decreased dramatically over 48 h from about 6.5 $\log_{10}$ cfu/ml to below detectable limits (<3 $\log_{10}$ cfu/ml). Elimination of Coliforms from the LMW within 48 h was only achieved through back-slopping. However, there was no apparent advantage in increasing the backslop percentage from 20 to 33 or 42%. The level of starch and sugars decreased ($P<0.05$), whereas lactic acid and acetic acid concentrations increased ($P<0.001$) during 48 h-fermentation. This study demonstrated that LAB were capable of producing organic acids, predominantly lactic acid, from the reducing sugars present in the wheat. Back-slopping accelerated the acidification of the LMW and was essential to eliminate the coliform bacteria within the 48-hour steeping period.

INTRODUCTION

The effects on feed intake, growth performance and gastrointestinal 'ecophysiology' of pigs of feeding fermented liquid diets have been the subject of recent reviews by Jensen and Mikkelsen (1998) and Scholten et al. (1999). Fermented liquid feed (FLF) is characterised by high numbers of lactic acid bacteria (circa 10^9 cfu/ml), a low pH (3.8 to 4.2) and a high concentration of lactic acid (>150 mmol/l) (Geary et al., 1996). Concerns have been expressed that the fermentation of complete diets could lead to the production of protein fermentation products, such as biogenic amines that can make the diet unpalatable and reduce feed intake (Brooks et al., 1999). An alternative strategy would be to ferment the

carbohydrate fraction of the diet separately and combine the carbohydrate component with the protein components immediately before feeding (Rijnen and Scholten, 1998).

There could be practical advantages from fermenting the carbohydrate rich (e.g., cereals) component separately. First, pH may be reduced more rapidly as cereals have a lower buffering capacity than compound feed. Secondly, there would be less risk of producing undesirable protein breakdown products, like ammonia and biogenic amines which are harmful for the gastrointestinal mucosa (Visek, 1972, 1978), due to the lower protein content of the material being fermented. Thirdly, cereals have a more constant composition than compound feed. This could make it easier to develop a starter culture for cereals than for more complex compound feed, which in turn could result in a more controllable fermentation. Finally, fermenting a cereal that could be used as a component in all the diets used on a pig farm unit would have practical advantages. Less storage capacity would be needed, in contrast with the fermentation of compound feed where each diet used would have to be fermented and stored separately.

Wheat was chosen as the substrate for fermentation in this study as it is commonly included as an energy source in diets for all categories of pig and is the most common cereal in Europe and other temperate countries. Lactic acid bacteria (**LAB**) have complex growth requirements and require nitrogen and B-vitamins in sufficient quantities. However, milled whole-grain wheat has been found to contain all the necessary nutrient requirements to support the growth of LAB (Kumar and Raccach, 1996; Hofvendahl and Hahn-Hagerdal, 1997a,b).

The production of traditional fermented cereal products, for human consumption, involves mixing the milled grain with water and allowing it to ferment overnight or longer, usually at ambient temperature (Odunfa, 1985; Adams and Nicolaides, 1997). A succession evolves within the epiphytic microflora during the steeping period. There is a short period of growth by fungi and bacteria, followed by a lactic acid and alcohol fermentation (Odunfa, 1985). The lactic fermentation inhibits pathogenic and spoilage organisms by several mechanisms, including production of organic acids, hydrogen peroxide, and bacteriocins, and by lowering the pH and oxidation-reduction potential (Lindgren and Dobrogosz, 1990).

A portion of a previous successful fermentation is often retained as an inoculum for the next batch. This is known as 'back-slopping' (Salovaara, 1998). In practice, back-slopping can involve the retention of a specified quantity of previously fermented product in the tank and mixing in fresh substrate or the addition of a portion of the fermented product to a fresh tank of substrate to act as an inoculum. This allows for the gradual selection of LAB and an accelerated fermentation (Nout et al., 1989). LAB species that have been isolated from traditionally fermented cereals include *Lactobacillus plantarum* and *Pediococcus*

pentosaceus (Nche et al., 1994). Therefore, these bacteria were used in the starter culture tested in this experiment.

The objectives of the study reported here were to investigate the changes in chemical and microbial composition of milled wheat fermented either by epiphytic microorganisms or following inoculation with a starter culture and to investigate the potential use of the back-slopping technique to optimise the fermentation process.

MATERIAL AND METHODS

Experimental design and treatments

The study was arranged as a randomised block experiment with five wheat preparation treatments and three replicates. The five treatments were:

Treatment 1 (Control)

Liquid milled wheat (LMW) was prepared by mixing 5 kg wheat, which had been hammer-milled through a 3 mm sieve, with 15 kg water (30 °C) to produce a mixture containing 210 g DM/kg product.

Treatment 2 (0% BS)

LMW (prepared as in Treatment 1) was mixed for 30 minutes and then inoculated with both 10 ml of *Lactobacillus plantarum* (PC-81-11-06, Alltech Inc., Kentucky, USA) and 10 ml of *Pediococcus pentosaceus* (SHCM-02, Alltech Inc., Kentucky, USA). The *Lb. plantarum* and *P. pentosaceus* starter cultures had previously been subcultured overnight in individual 10 ml MRS broth at 30 °C. The combined 2 x 10ml liquid starter culture gave a final concentration of approximately 6 $\log_{10}$ cfu/ml liquid feed of each organism. The mixture was stirred continuously for a further 15 minutes to ensure a homogenous distribution of the starter culture.

Treatment 3 (20% BS), Treatment 4 (33% BS) and Treatment 5 (42% BS)

LMW was prepared by mixing 5 kg wheat, which had been hammer-milled through a 3 mm sieve, with 15 kg water (30 °C) to produce a mixture containing 210 g DM/kg product. To this was added 5, 10, or 15 kg of the pre-fermented wheat; equivalent to 20%, 33% and 42% of the final quantity respectively. The pre-fermented wheat was prepared 48 hours prior to addition to the LMW by mixing 10 kg milled wheat with 30 kg water (1:3 wt/wt) and inoculating with the starter culture as used in Treatment 2.

Procedure

Five, 45-litre capacity lockable PVC storage tanks were used to store the LMW during the 48-hour trial period. Treatments were randomly assigned to storage tanks and were replicated three times. The storage tanks were housed in a temperature-controlled room set at 24 ± 1 °C. Loss due to evaporation was minimised by covering the tanks with lids.

One hour before the start of each storage period (Time = 0, T_0) the treatments were prepared and mixed. Throughout the storage period each tank was mixed automatically, for two minutes every two hours, by an overhead mixer and stirring bar. The pH-value and temperature were measured and recorded every 2 hours automatically by a Consort-Controller with appropriate electrodes.

Samples were removed aseptically from each fermentation tank at times 0, 24 and 48 hours (T_0, T_{24}, T_{48} respectively) after the contents of the tanks had been stirred for two minutes. Samples for chemical analysis were frozen immediately and kept at –20 °C until analysis. Samples for microbial analysis were transported directly to the laboratory under cooled conditions (3-5 °C) and processed within one hour of sampling.

Between replicates tanks were washed with hot water and sanitiser (Pipeclean®, The Netherlands), rinsed with hot water and subsequently dried.

Chemical analysis

Dry matter, sugars, total carbohydrates, lactic acid, acetic acid and ethanol in the LMW samples were determined as follows. Dry matter was estimated at each sampling time by drying the samples in an oven (103 °C) to a constant weight (method: ISO 6469 / NEN 3332). Reducing sugars were determined by the Luff-Schoorl method (Directive 71/250/EEC: OJ No. L155, 12.7.71, p21). The results were expressed as glucose (%). Total carbohydrates were determined in a three-step process. Five grams of LMW were boiled to extract the starches and sugars. The starch was transformed into soluble carbohydrates by pancreatic amylase (Sigma-Aldrich, Netherlands) and the sugars subsequently quantified by the Luff-Schoorl method. Starch was calculated as the difference between reducing sugars and total carbohydrates. Organic acids were determined by capillary electrophoresis (Soga and Ross, 1999). Samples were prepared by filtration through a 0.22 μm filter and subsequently diluted, as necessary, before analysis. Acids were separated in a capillary under the influence of a potential difference and detected with an UV- detector at 254nm wavelength. Ethanol was measured in the supernatant fraction of the LMW by HPLC using a Spectra Physics SP8000 liquid chromatograph equipped with an Erma-ERC 7510 refractive index (RI) detector maintained at 40°C. The column used was an Aminex HPX-87H column (300x7.8 mm; BioRad Labs, Richmond, California, USA) protected with a guard column

(50x4.6 mm) packed with AG 50 W-X4 (H^+, 400 mesh; BioRad Labs). Elution was performed with 5mM sulphuric acid at 65°C and a flow rate of 0.6 ml/min (Middelhoven, 1998). Due to the sampling technique, ethanol concentration could not be related back to dry matter.

Quantification of indicator microbial populations

Microbial analysis of the LMW samples was conducted using selective media (Oxoid, Basingstoke, UK). Microbiological counts were determined from decimal dilutions of LMW samples in Maximum Recovery Diluent (MRD) (1ml sample: 9 ml MRD). LAB were enumerated on double-layered pour plates of MRS agar and incubated aerobically for 3 days at 30°C. Coliforms were enumerated on MacConkey agar (MAC) (spread plate technique) after incubating aerobically for 24 hours at 37°C. Yeast were enumerated on Rose Bengal Chloramphenicol agar (RBCA) (spread plate technique) after incubating aerobically for 3 days at 25°C.

Statistical analysis

Bacterial counts were log transformed to fit a normal distribution prior to statistical analysis. Results of the chemical analyses were expressed as grams per kilogram dry weight (g/kg DM). Experimental data were analysed using a general linear model analysis of variance (GLM). Significant differences between treatment means ($P<0.05$) were compared by Tukey's HSD test (Zar, 1984).

The Control and inoculated fermentations (0% BS) were analysed separately to investigate the influence that addition of the mixed starter culture had on the fermentation. Relationships between measured variables were determined using Pearson's product moment correlation co-efficient. The statistical analyses were undertaken using Minitab v. 10.2 (Minitab Inc., Pennsylvania, USA, 1994).

RESULTS

Effect of the addition of a starter culture

The effect on the fermentation characteristics of LMW of adding an inoculum is shown in Table 1. The inclusion of the inoculant increased the total lactic acid bacteria (**LAB**) population at T_0 compared with the uninoculated control ($P<0.01$). LAB counts increased rapidly over the 48-hour steeping period but did not differ ($P>0.05$) between the control and inoculated fermentations at the 24 and 48 hour sampling times.

Coliform population numbers in both the uninoculated and inoculated wheat increased ($P<0.001$) over the first 24 hours of steeping (Table 1). Inclusion of the inoculum resulted in a lower coliform population after 48 hours compared with the Control fermentation ($P<0.01$). Addition of the inoculant had no influence on the yeast population at any sampling time.

Lactic acid concentration increased with time but did not differ significantly between the control and inoculated treatments ($P>0.05$). Acetic acid concentration increased more rapidly and to a higher level in inoculated feed. Inoculated feed had in a greater acetic acid concentration ($P<0.001$) than the Control after 24 and 48 hours ($P<0.05$) (Table 1). There was a decrease in pH ($P<0.001$) over time in both the Control and inoculated (0% BS) LMW (Table 1).

Concentrations of dry matter, total carbohydrates, reducing sugars and lactic acid were not affected by addition of the inoculum but were significantly affected by steeping time ($P<0.05$).

Table 1. Comparison of microbial (log_{10} cfu/ml) and chemical (g/kg DM) composition of liquid milled wheat with (0%BS) and without (Control) lactic acid bacteria inoculant in liquid wheat at T_0, T_{24} and T_{48} after start of steeping period

	T_0		T_{24}		T_{48}			Significance		
	Control	0% BS	Control	0% BS	Control	0% BS	s.e.d	Treatment	Time	Int.[1]
Lactic acid bacteria	4.3[a]	6.4[b]	9.3[a]	9.3[a]	8.5[a]	8.6[a]	0.12	***	***	***
Coliforms	3.0[a]	3.1[a]	6.2[a]	6.4[b]	6.7[a]	5.8[b]	0.03	***	***	***
Yeasts	3.9[a]	3.9[a]	5.5[a]	5.3[a]	7.1[a]	7.4[a]	0.19		***	
Dry matter (g/kg)	209[a]	212[a]	220[a]	213[a]	189[a]	186[a]	7.0		*	
Reducing sugars	60.8[a]	61.6[a]	59.0[a]	64.4[a]	7.1[a]	10.9[a]	3.43		***	
Total carbohydrates	743[a]	755[a]	714[a]	727[a]	650[a]	665[a]	16.2		**	
Lactic acid	0.0[a]	0.0[a]	32.0[a]	28.4[a]	54.1[a]	55.7[a]	2.88		***	
Acetic acid	0.0[a]	0.0[a]	3.4[a]	6.2[b]	4.8[a]	6.6[b]	0.35	**	***	*
PH	5.9[a]	5.8[a]	3.9[a]	4.2[b]	3.7[a]	3.8[a]	0.03	**	***	**

[a, b] Within time block, means with different superscript in the same row are significantly different ($P<0.05$).
[1] Interaction = Treatment x Time.

Effect of back-slopping

The effect of back-slopping at different levels (0%, 20%, 33% and 42%) and time (0, 24 and 48) on the microbial populations of liquid wheat is shown in Table 2, with the main effects of back-slopping and time shown in Table 3.

At time 0 increasing backslop % resulted in a proportional increase in LAB, Coliforms and yeasts. Over the 48 h steeping period both the LAB and yeast populations tended to increase. The most noticeable effect was on the coliform bacteria. Coliform populations were the same at 0 and 24 h but decreased ($P<0.001$) between 24 and 48 h. Back-slopping reduced the coliform populations compared with the 0% backslop treatment but tended to increase with backslop %. A similar pattern was found for yeasts.

In the backslop treatments, coliform populations decreased dramatically over 48 hours from about 6.5 $\log_{10}$ cfu/ml to below detectable limits (< 3 $\log_{10}$ cfu/ml) (Table 2). In the 0% BS, Coliforms increased over the initial 24 hours of steeping with a slight reduction in the subsequent 24 hours. Large numbers of Coliforms were transferred from the pre-fermented wheat through the back-slopping process to each of the backslop treatments. The lowest coliform numbers, after 24 hours, were found in the 20% BS treatment ($P<0.001$). Coliform populations decreased from 5.7 to 1.5 $\log_{10}$ cfu/ml, in the 24 to 48 hour period (Table 3). The addition of pre-fermented wheat reduced the coliform populations from 5.1 to approximately 4.0 $\log_{10}$ cfu/ml. Yeasts, like the bacteria, were transferred across in high numbers in the back-slopping process. Back-slopping increased ($P<0.001$) the yeast populations from 5.5 (0% BS) to about 7.2 $\log_{10}$ cfu/ml (Table 3).

Table 2. Microbial content (log_{10} cfu/ml) and chemical composition (g/kg DM) of liquid milled wheat with increasing concentration of backslop at T_0, T_{24} and T_{48} after start of the steeping period

	T_0				T_{24}				T_{48}				
Backslop (%)	0	20	33	42	0	20	33	42	0	20	33	42	s.e.d
LAB	6.35^{b}	8.37^{a1}	8.55^{a1}	8.63^{a1}	9.25^{b}	8.56^{a12}	8.64^{a12}	8.78^{a1}	8.56^{a}	8.68^{a2}	8.77^{a2}	8.68^{a1}	0.06
Coliforms	3.09^{b}	6.49^{a}	6.47^{a}	6.69^{a}	6.43	5.13	5.44	5.73	5.81^{b}	$<3.0^{a}$	$<3.0^{a}$	$<3.0^{a}$	0.06
Yeasts	3.87^{b}	6.42^{a}	6.53^{a}	6.57^{a}	5.26^{b}	7.42^{a1}	7.49^{a1}	7.52^{a1}	7.42^{a}	7.56^{a1}	7.61^{a1}	7.62^{a1}	0.16
Dry Matter (g/kg)	212^{a1}	211^{a1}	207^{a1}	202^{a1}	213^{a1}	186^{b1}	193^{ab1}	193^{ab1}	186^{a}	140^{b}	188^{a1}	181^{a1}	7.7
Carbohydrates	755^{1}	736^{1}	711^{1}	718^{1}	727^{b12}	639^{a1}	651^{a1}	649^{a1}	700^{a2}	586^{b}	624^{ab}	640^{ab}	16.5
Reducing sugars	61.2^{a1}	49.0^{ab}	48.2^{ab}	28.0^{b}	64.3^{1}	7.20^{a1}	10.4^{a1}	5.20^{a1}	10.8^{a}	11.9^{a1}	5.30^{a1}	5.50^{a1}	4.48
Lactic acid	0.00^{b}	16.6^{a}	19.6^{a}	23.9^{a}	28.4^{b}	55.5^{a}	50.4^{a}	57.6^{a1}	54.5^{a}	85.2^{b}	65.6^{a}	65.8^{a1}	4.11
Acetic acid	0.00^{a}	1.91^{ab}	2.89^{b1}	3.49^{b1}	6.21^{a1}	4.70^{a}	4.58^{a2}	1.76^{b}	6.52^{a1}	7.40^{a}	2.93^{b12}	3.43^{b1}	0.50
PH	5.83	4.75	4.52	4.33	4.19^{c}	3.72^{a1}	3.74^{ab1}	3.78^{b1}	3.82^{a}	3.65^{b1}	3.69^{ab1}	3.69^{ab1}	0.04

[1,2,3] Within treatment, means with different superscript in the same row are different ($P<0.05$).
[a,b,c] Within time blocks, means with different superscript in the same row are different ($P<0.05$).

Table 3. Least square means of microbiological population (log_{10} cfu/ml) in fermented liquid milled wheat with different levels of back-slopping (0, 20, 33 and 42%) measured at three time intervals (0, 24 and 48 hours)

	Backslop (%)					
	0	20	33	42	s.e.d.	*P*
Lactic acid bacteria	8.1[a]	8.5[b]	8.7[b]	8.7[b]	0.07	***
Coliforms	5.1[a]	3.9[b]	4.0[b]	4.1[c]	0.06	***
Yeasts	5.5[a]	7.1[b]	7.2[b]	7.2[b]	0.18	***

	Time (h)				
	0	24	48	s.e.d.	*P*
Lactic acid bacteria	8.0[a]	8.8[b]	8.7[b]	0.06	***
Coliforms	5.7[a]	5.7[a]	1.5[b]	0.05	***
Yeasts	5.9[a]	6.9[b]	7.6[c]	0.15	***

[a,b,c] Within rows, means with different superscript are different (P<0.05).

The effect treatment on the chemical composition of liquid milled wheat is shown in Tables 2 and 4. Dry matter (DM) was reduced by fermentation at different rates depending on the treatment. In 0% BS there was no loss of DM during the initial 24 hours of steeping, but a 12.7% decrease (P<0.001) in the subsequent 24 hours. The greatest reduction in DM (33.6%) was found in the 20% BS treatment. There was no significant difference in the DM concentration in the other backslop treatments (Table 4).

Both steeping time and backslop % had a significant effect on the concentration of carbohydrates in the fermentations (Table 2). The carbohydrate concentration reduced at the greatest rate (P<0.001) over the first 24 hours in the backslop treatments whilst the rate of decline slowed in the second 24 hours. The greatest reduction in total carbohydrates, 150 g total carbohydrates/kg DM, was found in the 20% BS fermentations compared with 55, 87 and 78 g/kg DM in the 33, 42 and 0% BS fermentations, respectively (Table 2). Therefore, with increasing backslop addition there was a smaller reduction in total carbohydrates utilised in the fermentation process. The total carbohydrates in the control treatment reduced by 28 g/kg DM in the first 24 hours, and 27 g/kg DM in the subsequent 24 hours (Table 2).

The starch fraction of the total carbohydrates present in the LMW was reduced (P<0.05) over the 48 hours of steeping. Both back-slopping and time had an effect on the concentrations of reducing sugars in the fermentations. There was a reduction (P<0.001) in the reducing sugar concentration in all treatments.

Table 4. Least square means of chemical composition (g/kg DM) and pH in fermenting liquid milled wheat with different levels of back-slopping (0, 20, 33, 42%) measured at three time intervals (0, 24 and 48 hours)

	Backslop (%)					
	0	20	33	42	s.e.d.	*P*
Dry matter (g/kg)	204^{a}	179^{b}	196^{ab}	192^{ab}	8.2	*
Total carbohydrates	716.2^{a}	647.7^{b}	662.3^{b}	668.9^{b}	17.6	**
Starch	670.1^{a}	624.3^{a}	640.5^{a}	656.2^{a}	16.7	Ns
Reducing sugars	45.6^{b}	23.2^{a}	21.3^{a}	12.9^{a}	4.8	***
Lactic acid	28.3^{b}	54.0^{a}	45.3^{a}	49.1^{a}	4.39	***
Acetic acid	4.2^{a}	4.7^{a}	3.5^{ab}	2.9^{b}	0.54	**
PH	4.6^{b}	4.0^{a}	4.0^{a}	3.9^{a}	0.04	***

	Time (h)				
	0	24	48	s.e.d.	*P*
Dry matter (g/kg)	208^{a}	196^{a}	174^{b}	7.1	***
Total carbohydrates	729.7^{a}	665.6^{b}	624.2^{c}	15.3	***
Starch	683.4^{a}	644.3^{b}	615.6^{b}	16.7	**
Reducing sugars	46.7^{a}	21.7^{b}	8.9^{c}	4.15	***
Lactic acid	15.2^{a}	48.3^{b}	69.0^{c}	3.80	***
Acetic acid	2.1^{b}	4.4^{a}	5.2^{a}	0.46	***
PH	4.9^{a}	3.9^{b}	3.7^{c}	0.03	***

^{a,b,c} Within rows, means with different superscript are different (P<0.05).

Lactic acid concentration dramatically increased (P<0.001) with steeping time for all treatments. The lactic acid concentration in the 20% BS treatment after 48 hours (85.2 ± 4.11 g/kg DM) was higher (P<0.001) than all the other treatments (Table 2). Acetate concentrations increased (P<0.001) in all treatments over the first 24 hours but did not

change significantly thereafter. An increase in the concentration of backslop used resulted in a decrease ($P<0.01$) in the concentration of acetic acid (Table 4).

The pH of the LMW at T_0 was inversely related to the backslop %. At 48 h the 20% BS treatment had a lower pH than the other treatments. Back-slopping reduced the pH compared with the 0% BS treatment ($P<0.001$; Table 4). The time taken for the fermentation to reduce to pH-value 4.0 was influenced by the treatment. In both the 20% BS and 33% BS treatments pH-value 4.0 was achieved within 8 hours whilst the Control and 42% BS treatments needed 20 hours and 0% BS treatment needed 34 hours.

Ethanol was measured in the supernatant of the LMW and hence cannot be related back to dry matter, although a strong relationship would exist with overall ethanol content. Ethanol concentration increased 10-fold in the second 24 hour period of the fermentation in both the control and 0% BS treatments, from approximately 20 to 200 mmol/l (Figure 1). Initial ethanol concentration in the 20, 33 and 42% BS tanks was positively correlated with the quantity of pre-fermented wheat added at T_0.

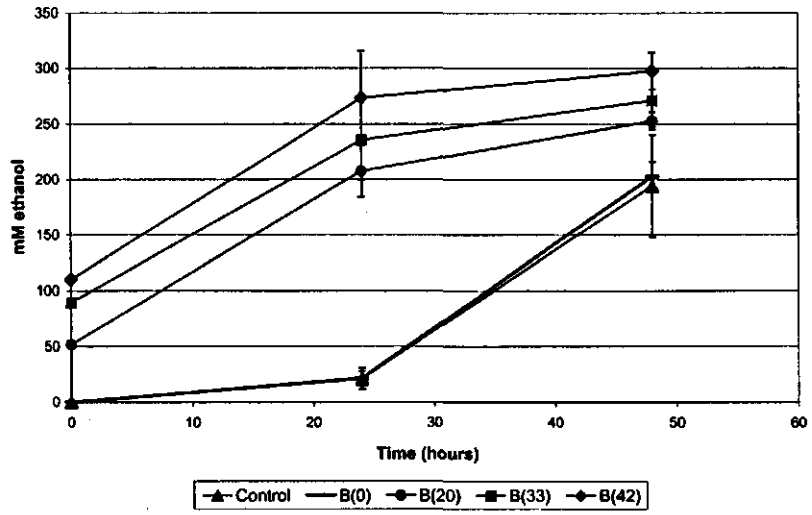

Figure 1. Contents in ethanol concentration (mmol/l) in the supernatant of the LMW during fermentation with and without different inclusions of backslop (B: 0, 20, 33 and 42%) and in the uninoculated fermentation (Control)

DISCUSSION

The composition, digestibility and availability of wheat make it an usable raw material for inclusion in pig diets. There have been very few studies on the fermentation of liquid milled wheat for pigs, although liquid wheat starch, a liquid co-product originating from human food industry, is commonly used in liquid feeding practice (Rijnen and Scholten, 1998; Scholten et al., 1999). The limited information available, suggests that LAB can utilise wheat successfully as a substrate without nutritional supplementation (Hofvendahl and Hahn-Hagerdal, 1997a). The current study confirmed that LAB can ferment wheat successfully and demonstrated that during steeping liquid milled wheat undergoes a rapid fermentation. The fermentation results in a product that has a large population of LAB and yeasts, a high lactic acid concentration and a low pH. This process was in the present study not dependent on the inclusion of a starter culture.

The initial pH of the inoculated LMW was 5.8 and declined to 3.8 after 48 h fermentation. The reduction of pH is much more rapid in the liquid milled wheat compared with the fermentation of liquid compound diets. Scholten et al. (2001b) fermented a liquid grower diet and liquid finisher diet under the same conditions as were used in the current study. They found that the diets decreased from their initial pH values of 5.7 and 5.6 respectively, to pH-values of 4.7 and 4.4 after 48 hours storage and to 3.8 and 3.9 after 148 h storage. In general, it is assumed that a pH-value below 4.5 is needed to obtain a diet without Coliforms (Russell et al., 1996). Therefore, individually fermented raw materials might be more successful for application in fermented diets than completely compound diets.

In the current study, the concentration of lactic acid, approximately 55 g/kg DM in the 48-h fermented LMW product, was lower than that reported by Scholten et al. (2001b) for liquid grower and liquid finisher diets (129 and 92 g/kg DM, respectively). The rapid production of lactic acid and low pH-value of the LMW may have resulted in the inhibition of the LAB, thereby reducing further acid production (Vandevoorde et al., 1992).

In the study reported here, DM decreased by 20 and 26 g/kg for uninoculated (Control) and inoculated (0% BS) LMW respectively, over the 48h fermentation period. This is similar to the decline in DM reported in liquid grower diet and liquid finisher diet (21.1 and 23.8 g/kg) during a six-day storage period under identical conditions (Scholten et al., 2001a). Based on limited information, CVB (2000) has assumed that there is a loss of 8% lactic acid, 50% acetic acid and 100% ethanol due to volatilisation during the procedure for determination of dry matter content. In the current study, the ethanol lost during the drying process could not be estimated, as the sampling technique did not allow for DM content. However, it is obvious that during fermentation the concentration of ethanol increased rapidly (Figure 1). Therefore,

it is reasonable to assume that a proportion of the dry matter lost was likely to have been due to the production of ethanol and CO_2 by the yeast during the steeping process (Barnett et al., 1990).

The fermentation process will change the type of energy available to the pig from the diet. During storage, α- and β-amylases degrade wheat starch to dextrins and maltose (Lynch et al., 1962; Pomeranz, 1992). In this study, on DM-base the total carbohydrate content was reduced by 93 and 90 g/kg DM for the uninoculated (Control) and inoculated (0% BS) LMW respectively. The relative decrease in carbohydrate content was 12-13% after 48 h. This is comparable with the decrease in carbohydrate content of about 14% after 72 h storage of liquid grower diet reported by Scholten et al. (2001a), but lower than the 21% decrease observed in a liquid finisher diet (Scholten et al., 2001a).

An important aim of fermenting feed components is the elimination of the Coliforms. In this study elimination of Coliforms within 48 h in the LMW was only achieved through back-slopping. However, there was no apparent advantage in increasing the backslop percentage from 20 to 33 or 42%.

The relatively slow growth of the LAB in the inoculated LMW (0% BS) compared with the Control LMW may have been due to the liquid inoculum being prepared in a glucose based medium. The major carbohydrate available to the LAB in the LMW would have been maltose and dextrins as products of wheat starch degradation (Lynch et al., 1962; Pomeranz, 1992). Bonestroo et al. (1992) described an increase in lag phase and concomitant reduction in lactic acid production when a starter culture was added to an alternative carbon source. They ascribed this to the presence of low levels of specific permeases and / or hydrolysing enzymes. It was evident that these enzymes could be induced, as lactic acid production and LAB growth were observed after a prolonged period of time. In order to optimise the performance of a starter culture it may be beneficial to prepare them using the same carbohydrate sources that they are intended to use in the fermentation mixture.

This study demonstrated that the LAB were capable of producing organic acids, predominantly lactic acid, from the reducing sugars present in the wheat. The organic acids lowered the pH of the LMW and produced a hostile environment for the Coliforms. The inclusion of a starter culture resulted in a lower coliform population at the end of the steeping period. Back-slopping accelerated the acidification of the LMW and was essential to eliminate the Coliforms within the 48-h steeping period. Furthermore, lactic acid may have other benefits to the pig in addition to providing an energy source (Brooks, 1999; Scholten et al., 1999).

The use of a fermented cereal as a component in diets could enable producers that do have not the capacity to ferment a range of different diets to gain the benefits of feeding fermented liquid feed. Additional research, however, to the effect of the addition of fermented wheat to pig diets on the growth performance and animal health is needed to conclude more firmly about the possibilities to use fermented wheat in pig diets.

ACKNOWLEDGEMENTS

The authors wish to gratefully acknowledge Dr. Alfons Stams and Dr. Caroline Plugge at the Department of Microbiology (Wageningen University) for their considerable help, advice and use of their facilities.

REFERENCES

Adams, M.R., Nicolaides, L., 1997. Review of the sensitivity of different foodborne pathogens to fermentation. Food Control 8, 227-239.

Barnett, J.A., Payne, R.W., Yarrow, D., 1990. Yeasts: Characteristics and identification. 2nd ed. Cambridge University Press, Cambridge, UK.

Bonestroo, M.H., Kusters, B.J.M., de Wit, J.C., Rombouts, F.M., 1992. Glucose and sucrose fermenting capacity of homofermentative lactic acid bacteria used as starters in fermented salads. Int. J. Food Microbiol. 15, 365-376.

Brooks, P.H., 1999. The potential of liquid feeding systems. In: Lyons, T. P., Cole, D. J. A., (eds). Concepts in pig science. Nottingham University Press, Nottingham, pp. 81-98.

Brooks, P.H., Moran, C., Beal, J.D., 1999. Liquid feeding of pigs: Potential for reducing environmental impact and for improving productivity and food safety. In: Lyons, T. P., Jacques, K. A., (eds). Biotechnology in the feed industry: Proceedings of Alltech's 15th annual symposium. Nottingham University Press, Nottingham, pp. 111-129.

CVB, 2000. Gegevens over chemische samenstelling, verteerbaarheid en voederwaarde van voedermiddelen. Centraal Veevoederbureau, Lelystad, The Netherlands.

Geary, T.M., Brooks, P.H., Morgan, D.T., Campbell, A., Russell, P.J., 1996. Performance of weaner pigs fed *ad libitum* with liquid feed at different dry matter concentrations. J. Sci. Food. Agric. 72, 17-24.

Hofvendahl, K., Hahn-Hagerdal, B., 1997a. L-lactic acid production from whole wheat hydrolysate using strains of lactobacilli and lactococci. Enzyme and Microbial Technology 20, 301-307.

Hofvendahl, K., Hahn-Hagerdal, B., 1997b. The effect of nutrients and a-amylase inactivation on the fermentative lactic acid production in whole wheat flour hydrolysate by *Lactococcus lactis* subsp. *lactis* ATCC 19435. Cereals: Novel uses and processes. Plenum Press, New York, pp. 219-227.

Jensen, B.B., Mikkelsen, L.L., 1998. Feeding liquid diets to pigs. In: Garnsworthy, P. C., Wiseman, J., (eds). Recent advances in animal nutrition 1998. Nottingham University Press, Thrumpton, Nottingham, pp. 107-126.

Kumar, G., Raccach, M., 1996. The effects of temperature and manganese on the natural fermentation of whole wheatflour. Food Microbiol. 13, 149-157.

Lindgren, S., Dobrogosz, W.J., 1990. Antagonistic activities of lactic acid bacteria in food and feed fermentations. FEMS Microbiol. Rev. 87, 149-164.

Lynch, B.T., Glass, R.L., Geddes, W.F., 1962. Grain storage studies. 32. Quantitiative changes occuring in the sugars of wheat deteriorating in the presence and absence of molds. Cereal Chemistry 39, 256-262.

Middelhoven, W.J., 1998. The yeast flora of maize silage. Food Technol. Biotechnol. 36, 7-11.

Nche, P.F., Nout, M.J.R., Rombouts, F.M., 1994. Gas production by *Clostidium perfringens* as a measure of the fermentability of carbohydrates and processed cereal-legume foods. Food Microbiol. 11, 21-29.

Nout, M.J.R., Rombouts, F.M., Havelaar, A., 1989. Effect of accelerated natural lactic fermentation of infant food ingredients on some pathogenic microorganisms. Int. J. Food Microbiol. 8, 351-361.

Odunfa, S.A., 1985. African fermented foods. In: Wood, B. J. B., (ed.) Microbiology of fermented foods, Vol. 2. Elsevier, New York, pp. 155-192.

Pomeranz, Y., 1992. Biochemical, functional, and nutritive changes during storage. In: Sauer, D. B., (ed.). Storage of cereal grains and their products. American Association of Cereal Chemists Inc., St. Paul, Minnesota, pp. 55-141.

Rijnen, M.M.J.A., Scholten, R.H.J., 1998. Fermentation of liquid diets and by-products during Storage. Report P1.211(in Dutch, with English summary). Praktijkonderzoek Varkenshouderij, Rosmalen, The Netherlands.

Russell, P.J., Geary, T.M., Brooks, P.H., Campbell, A., 1996. Performance, water use and effluent output of weaner pigs fed ad libitum with either dry pellets or liquid feed and the role of microbial activity in the liquid feed. J. Sci. Food Agric. 72, 8-16.

Salovaara, H., 1998. Lactic acid bacteria in cereal-based products. In: Salminen, S., von Wright, A., (eds). Lactic acid bacteria: Microbiology and functional aspects., 2nd edition, Marcel Dekker Inc., New York, pp. 115-137.

Scholten, R.H.J., van der Peet-Schwering, C.M.C., Verstegen, M.W.A., den Hartog, L.A., Schrama, J.W., Vesseur, P.C., 1999. Fermented co-products and fermented compound diets for pigs: a review. Anim. Feed Sci. Technol. 82, 1-19.

Scholten, R.H.J., Rijnen, M.M.J.A., Schrama, J.W., Boer, H., Vesseur, P.C., den Hartog, L.A., van der Peet-Schering, C.M.C., Verstegen, M.W.A., 2001a. Fermentation of liquid coproducts and liquid compound diets: Part 1. Effects on chemical composition during a 6-day storage period. J. Anim. Physiol. Anim. Nutr. 85, 111-123.

Scholten, R.H.J., Rijnen, M.M.J.A., Schrama, J.W., Boer, H., van der Peet-Schering, C.M.C., den Hartog, L.A., Vesseur, P.C., Verstegen, M.W.A., 2001b. Fermentation of liquid coproducts and liquid compound diets: Part 2. Effects on pH, acid binding capacity, organic acids and ethanol during a 6-day storage period. J. Anim. Physiol. Anim. Nutr. 85, 124-134.

Soga, T., Ross, G.A., 1999. Simultaneous determination of inorganic anions, organic acids, amino acids and carbohydrates by capillary electrophoresis. Journal of Chromatography A 837, 231-239.

Vandevoorde, L., Woestyne, M. van de, Bruyneel, B., Christiaens, H., Verstraete, W., 1992. Critical factors governing the competitive behaviour of lactic acid bacteria in mixed cultures. In: Wood, B. J. B., (ed.) The lactic acid bacteria. Elsevier Applied Science, London, pp. 447-475.

Visek, W.J., 1972. Effects of urea hydrolysis on cell life-span and metabolism. Fed. Proc. Fed. Am. Soc. Exp. Biol. 31, 1178-1193.

Visek, W.J., 1978. The mode of growth promotion by antibiotics. J. Anim. Sci. 46, 1447-1469.

Weast, R.C., 1968. Handbook of Chemistry and Physics, 49th Edition, The Chemical Rubber Company, Cleveland.

Zar, J.H., 1984. Biostatistical analysis. 2nd ed. Prentice Hall (UK), London, U.K.

Chapter 5

FERMENTED WHEAT IN LIQUID DIETS: EFFECTS ON GASTROINTESTINAL CHARACTERISTICS IN WEANLING PIGLETS

Ronald H.J. Scholten[1,*], Carola M.C. van der Peet-Schwering[1], Leo A. den Hartog[1], Johan W. Schrama[2], Martin W.A. Verstegen[3]

[1] Research Institute for Animal Husbandry, Lelystad, The Netherlands

[2] Wageningen Institute of Animal Sciences, Fish Culture and Fisheries Group, Wageningen, The Netherlands

[3] Wageningen Institute of Animal Sciences, Animal Nutrition Group, Wageningen, The Netherlands

[*] Present address: Beuker Vochtrijke Diervoeders B.V., Doetinchem, The Netherlands

Submitted to:

Journal of Animal Science

ABSTRACT

The effects of adding fermented wheat to liquid diets on gastrointestinal characteristics in weanling piglets were studied. Gastrointestinal characteristics of 40, 28-d old weanling piglets were measured at the day of weaning (**d0**) and at day 4 (**d4**) and day 8 (**d8**) after weaning. Piglets were group-housed and fed twice a day. Feeding level was based on the average metabolic BW of piglets per group. Groups were fed a liquid diet with either 45% non-fermented wheat (**FERM_0**) or 45% fermented wheat (**FERM_45**). The other 55% of the diet was identical. To ferment wheat water of 30 °C was added to milled wheat, in a 1:2.2 ratio. Afterwards, wheat was fermented by naturally present microbes for 24 h using a residue of 20% fermented wheat for a previous batch as inoculum. The pH and contents of lactic acid, acetic acid, propionic acid and butyric acid were measured in the digesta of the stomach, three parts of the small intestine, ceacum and large intestine. In addition, changes in microbial populations in the digesta were studied during the period after weaning. Moreover, villous height, crypt depth and villous shape were studied in the small intestine. The microbial composition of FERM_45 was different from FERM_0, reflected by higher numbers of lactic acid bacteria (**LAB**), yeast, Coliforms and E.coli ($P<0.05$). However, in the stomach only the number of LAB and yeast was higher for piglets fed FERM_45 ($P<0.05$), at both d4 and d8. Piglets fed FERM_45 showed lower gastric pH ($P<0.05$) at d4, and higher gastric lactic acid content ($P<0.001$) at both d4 and d8. Piglets fed FERM_45 showed in the first part of the small intestine higher villous height ($P<0.01$) at d8, and higher villous to crypt ratio ($P<0.001$) at both d4 and d8. Villous shape tended ($P<0.10$) to be favorable for piglets fed FERM_45. The present study indicates that feeding a partly fermented liquid diet to weanling piglets may be a concept to prevent undesirable changes in villous architecture post weaning.

INTRODUCTION

After weaning, villous shortening and crypt deepening in the small intestine of piglets often occurs within a few days (Makkink, 1993; Van Beers-Schreurs, 1996). Sufficient feed intake directly after weaning seems to prevent the undesirable changes of the morphology in the small intestine (Makkink, 1993; Pluske et al., 1997). Several authors suggest that diet formulation might influence gastrointestinal morphology, physiology and microbiology (e.g., Van Beers-Schreurs, 1996; Pluske et al., 1997; Jensen and Mikkelsen, 1998). One possible effect of diet formulation might be caused by presence of short chain fatty acids (**SCFA**) as hypothesized by Scholten et al. (1999). This is supported by the fact that villous height in piglets is positively correlated with contents of SCFA in the intestinal lumen (Nousiainen, 1991).

Fermented diets are characterized by a pH below 4.0 and high contents of SCFA (Scholten et al., 2001a,b). Current data on effects of feeding fermented diets to weanling piglets are based on research with completely fermented liquid compound diets (Russell et al., 1996; Jensen and Mikkelsen, 1998) rather than on research with liquid diets to which fermented carbohydrate rich feed ingredients (e.g., wheat) were added. Completely fermented liquid compound diets, however, had an undesirable effect on gain to feed ratio (Jensen and Mikkelsen, 1998). Recently, it was reported that feeding liquid diets supplemented with fermented carbohydrate rich feed ingredients favors both ADG and gain to feed ratio in weanling piglets (Scholten et al., 1999).

Scholten et al. (1999) hypothesized that fermented liquid diets have a positive influence on gastrointestinal health by a lower gastric pH, an increase in contents of SCFA, a reduction of microbial activity and improved mucosal architecture. The present study was carried out to examine effects of addition of fermented liquid wheat to a liquid diet on gastrointestinal characteristics of weanling piglets.

MATERIAL AND METHODS

Animals and Housing

The experiment was carried out in two trials. Per trial 20 weanling piglets were used, with an average weaning age of 27 d (SD = 1.5) and an average BW of 8.0 kg (SD = 0.08). Effects of diet composition were studied for a period of 8 days. Per trial, four piglets were dissected directly, eight piglets at day 4 after weaning and eight piglets at day 8 after weaning. The piglets were derived from Dutch Landrace, Finnish Landrace, and Dutch Large White rotational-bred sows and Great Yorkshire terminal boar. During the suckling period the piglets had free access to water and creep feed. At d3 after birth, male piglets were castrated. On the day of weaning, sows were removed from the piglets at about 0900. Afterwards, the piglets were transferred to the nursery room. The day of weaning is defined as d0. The Institutional Animal Care and Use Committee of the Wageningen Agricultural University approved the experimental protocols.

Piglets were housed in four pens of 2.65 x 0.75 m. The pens had fully slatted floors that consisted of 1.8-m plastic slats and 0.85-m metal tri-bar slats. The pens were situated in a room with computer-controlled heating and mechanical ventilation systems. In each pen four piglets were housed (two castrated males, two females). In the front of the pen a trough with four eating-places was present. Piglets were given *ad libitum* access to drinking water, which was supplied via nipples at the back of the pen.

Feeding

The work described in this paper was part of an larger investigation, which involved four dietary treatments: a control diet and three diets with fermented wheat ranging from 150 to 450 g/kg (Scholten et al., in prep.). The two dietary treatments used for this study were the control diet (**FERM_0**) and the diet to which 45% fermented wheat was added (**FERM_45**). Pens were randomly assigned to one of the two diets and to one of the two dissection days (d4 or d8 after weaning). Both experimental diets were similar in composition (Table 1), except for the wheat content. Non-fermented wheat was exchanged for fermented wheat, based on the weight after 24-h of fermentation. Furthermore, piglets were fed a diet without antibiotics, without added organic acids, and without pharmacological levels of copper and zinc (Table 1). To exclude possible effects of feed intake on gastrointestinal characteristics all piglets were fed restrictively. Piglets were fed according to the average metabolic BW per pen at a fixed level of maintenance requirements and an expected ADG of 150 g/d during the 8-day experimental period. The assumed ME requirements for maintenance was arbitrary assumed at 500 kJ/kg$^{0.75}$/day. The initial feeding level at d0 was 0.7 times the assumed maintenance requirements and increased daily with 0.1 times the assumed maintenance requirements. The used feeding levels and expected ADG were based on data of previous studies carried out at our research institute with the same type of piglets. Piglets received their daily feed in two similar portions at 0800 and 1600. The piglets were group-fed per pen, except for the feeding time before dissection. On the day of dissection the piglets were fed individually. To prevent fermentation in the through, feed refusals were removed 1.5 hours after the start of feeding. Total feed intake (feed offered minus feed refusals) was recorded daily.

Fermented wheat was prepared by adding 2.2 kg warm water (30 °C) to 1.0 kg hammer milled wheat (3 mm sieve) about 24 hours prior to feeding. More details about the fermentation process are described by Scholten et al. (in prep.). About one hour before feeding, the diets were prepared. Diet FERM_45 consisted of 55% supplementary feed to which 45% fermented wheat was added one hour before feeding. The supplementary feed was mixed with warm water in a water to feed ratio of 2.2:1. FERM_0 was prepared by adding warm water to the control diet, in a water to feed ratio of 2.2:1. All components (water, fermented wheat and control or supplementary feed) were weighed separately on a weight-scale. Just before feeding, the diets were stirred again thoroughly.

Table 1. Composition of experimental diets as fed (g/kg)

	FERM_0	FERM_45
Ingredient, g/kg		
Wheat		
Non-fermented	450	0
Fermented	0	450
Barley	94	294
Wheypowder delactosed	62	62
Soya beans, extracted, hypro	50	50
Sunflower seed, extracted	30	30
Lineseed expeller	40	40
Fish meal	34	34
Potato protein	10	10
Fat[1]	7.5	7.5
Limestone	7.1	7.1
Monocalciumphosphate	1.5	1.5
Lysine	6.1	6.1
Methionine	0.8	0.8
Threonine	0.8	0.8
Salt	1.0	1.0
Phytase	0.2	0.5
Vitamin and mineral premix[2]	5.0	5.0
Net Energy calculated (MJ/kg)[3]	9.68	9.68
Analyzed content, g/kg DM		
Crude Protein	189	188
Crude Fat	49	49
Ash	52	53
Starch	467	435
Sugars	53	33
Lactic Acid	6.2	27.0
Acetic Acid	1.3	4.5
Ethanol	0.9	5.4

[1] Mixture of 50% animal fat and 50% vegetable oil.

[2] Provided the following amount of vitamins and minerals per kilogram of complete diet (88% dry matter): vitamin A, 7.000 IU; vitamin D3, 1.400 IU; vitamin E, 12 mg; vitamin B2, 4 mg; panthotenic acid, 7 mg; niacin, 18 mg; biotin, 0.1 mg; folic acid, 1.5 mg; choline chloride, 250 mg; Fe as $FeSO_4 \cdot 7H_2O$, 100 mg; Cu as $CuSO_4 \cdot 5H_2O$, 15 mg; Zn as $ZnSO_4 \cdot H_2O$, 68 mg; Co as $CoSo_4 \cdot 7H_2O$, 0.25 mg; Mn as MnO_2, 24 mg; Se as $Na_2SeO_3 \cdot 5H_2O$.

[3] Calculation based on net energy formula of CVB (2000).

Dissection

Piglets were dissected at day 0 (d0; n = 8), or day 4 (d4; n = 16) or day 8 (d8; n = 16) after weaning. The dissections were divided over two trials. Piglets dissected at d0 were taken immediately from the sow. On the other days of dissection (d4 and d8), piglets were fed individually. For every piglet, dissection started exactly 1.5 hour after feeding. The first dissection started at 0830, the last at 1430. At d4 and d8, the first piglet to be dissected was randomly assigned. If the first piglet was FERM_0 than the second was FERM_45 and so on. At d4 and d8, per dietary treatment the whole pen (i.e., 4 piglets) was dissected.

Measurements Gastrointestinal Tract

Piglets were weighed directly before anesthesia. Under anesthesia, the abdomen was opened and the gastrointestinal tract (**GIT**) divided in seven segments using clamps and ligatures to prevent movement of digesta. The separated segments were: stomach (**ST**), small intestine I (**SI1**; 0.5 m distal to ligament of Treitz), small intestine II (**SI2**; 5.5 m to the ligament of Treitz), small intestine III (**SI3**; 0.5 m proximal to the ileo-caecal ligament), caecum (**CAE**), colon (**COL**) and rectum (**REC**). Immediately after removal of the GIT, segments were taken out, weighed, and digesta was collected. From SI1, SI2 and SI3 biopts of the gastrointestinal wall were taken and evaluated on morphological characteristics. Directly after taken out the GIT, the piglet was given a lethal injection with T61 (watery solution containing a combination of embutramide, mebezoniumiodide and tetracainehydrochloride; Hoechst Holland, Amsterdam, The Netherlands).

In the fresh digesta of every segment, pH was measured immediately after dissection. Thereafter, the digesta of segments was divided into samples for SCFA and microbial analyses. In digesta of all segments, the content of SCFA was analyzed. For microbial analyses only the segment ST, SI1, SI2 and SI3 were used. For microbial analyses the digesta were collected per piglet, put in an airtight bottle and flushed with N_2 for 30 s and placed in a refrigerator (4 °C) during the dissection day. At the end of the day, a pooled sample was made per treatment and put in an airtight bottle, flushed again with N_2, and sealed. Microbial analyses started within two hours after the last dissection, and were performed using appropriate dilution and culture techniques. The microbial groups that were analyzed, were lactic acid bacteria, yeasts, coliforms and *E.coli*. Samples were diluted in sterile phosphate buffered saline (**PBS**) with cystein (0.05% w/v). For lactic acid bacteria Rogosa Agar plates were used, incubated during 72 hours at 37 °C. To inhibit non-lactics, cooled agar 200 mg/l cycloheximide was added. Furthermore, 0.004% (w/v) bromocresolpurple was added to distinguish acid producing organisms from non-acid producing organisms. For Coliforms MacConkey agar plates were used, incubated 36 hours at

37 °C. For Yeasts Rose Bengal Chloramphenicol Agar Base was used, incubated during 72 hours at 37 °C. Prior to poring the sterile, cooled agar, 100 mg/l chloramphenicol was added to inhibit growth of non-yeast like organisms.

For SCFA analyses, the digesta of every segment was collected per piglet and stored individually. These samples were frozen quickly with dry ice and stored at –20 °C until analyses. The stored digesta were thawed at 4 °C and analyzed for contents of lactic acid, acetic acid, propionic acid and butyric acid.

The dissecting morphology of SI1, SI2 and SI3 was determined according to the method as described by Van Leeuwen et al. (1995). From stained sections, the villous length, crypt depth, villous to crypt ratio and index of mitosis (meta- and anaphases per 100 crypt cells) were determined according to reported procedures (Kik et al., 1990). Villous shape was determined according to the method of Mouwen (1972) and described by Van Leeuwen et al. (1995).

Feed Sampling and Analytical Procedures

The pH of the fermented wheat and the two liquid diets were measured (CONSORT, Belgium) every feeding time. The liquid fermented wheat and the liquid diets were sampled at d4 and d8. These samples were directly stored at –20 °C until analyses. Samples were subsequently analyzed on dry matter, starch, sugars, ethanol, lactic acid and acetic acid. All analyses were carried out in duplicate. The methods of sampling and analyzing are described by Scholten et al. (in prep.). In addition, on d4 and d8 fresh samples of the two liquid diets were taken for bacterial analysis and directly transported. Analysis for lactic acid bacteria, yeasts, Coliforms and *E.coli*, started within two hours after sampling, and were performed using appropriate dilution and culture techniques.

Growth Performance

The piglets were weighed individually at d0 (at weaning), d4 and d8 after weaning. Feed intake was recorded every feeding time. Because pen is the experimental unit, only two observations per dietary treatment and dissection day could be made. Therefore, no statistical analysis were carried out and the growth performance traits can only be used as indication.

Statistical Analyses

Data from piglets dissected at d0 were only used for reference values but not for statistical analyses. All statistical procedures were carried out using the General Linear Model procedure of SAS (SAS Inst. Inc., Cary, NC). The pH-value, level of lactic acid, acetic acid, propionic acid, and ethanol, villous height, crypt depth, villous to crypt ratio and index of mitosis were analyzed

using the using the following model, in which the pig was used as the experimental unit:

$$Y_i = \mu + B_i + D_j + T_k + (D_j * T_k) + e_{ijk}$$

where Y_i = dependent variable; μ = overall mean; B_i = trial (i = 1,2); Dj = dissection day (j = 4,8); T_k = treatment diet (k = FERM_0, FERM_45); e_{ijk} = error term.

Microbiological measurements and growth traits were tested with similar model as described above.

RESULTS

General

No health problems were observed during the experiment. Statistical analyses showed that no interaction occurred between dietary treatment and day of dissection. Therefore, in Tables 3, 4 and 5 the main effects of dietary treatment and day of dissection are given. Furthermore, it was observed that no dietary effect in SCFA concentrations and pH was observed in the CAE, COL and REC. Therefore in the Tables 4a+b, only the data from the stomach and separated parts of the small intestine are given.

Chemical composition of the diets

The pH of the liquid diets was 6.0 and 4.7 for FERM_0 and FERM_45, respectively. The analyzed nutrient composition of both diets is given in Table 1. The contents of crude protein, ash, crude fat were similar for both diets. In the FERM_45 diet, the contents of starch and sugar were lower, whereas the contents of lactic acid, acetic acid and ethanol were higher than in the FERM_0 diet.

Microbiology

The microbiology data of the diets are given in Table 2. The number of lactic acid bacteria, yeasts, total Coliforms and *E.Coli* was higher ($P<0.05$) for FERM_45 than for FERM_0.

The microbiology data of the GIT segments are given in Table 3. For SI1, no chymus could be taken for microbial analyses. Average over d4 and d8, the number of lactic acid bacteria and the number of yeasts in ST and SI2 of the piglets fed FERM_45 were higher than for piglets fed FERM_0 ($P<0.05$). Between the two treatments, no differences in the number of total Coliforms and *E.Coli* were observed in ST, SI2 and SI3 ($P>0.05$).

Table 2. Average number[1] (log_{10} cfu/g diet as fed) of total lactic acid bacteria, total yeasts, total coliforms and total *E.coli* in liquid diets with 0% (FERM_0) and 45% (FERM_45) fermented wheat

	FERM_0	FERM_45	SEM	P-value
Lactic acid bacteria	5.5	9.6	0.09	<0.001
Yeasts	2.9	3.9	0.22	0.032
Coliforms	6.4	9.6	0.12	<0.001
E.coli	5.2	8.4	0.19	<0.001

[1] Average based on three samples of each diet.

Physiology of the GIT

Mean pH and SCFA contents in the GIT are given in Tables 4a+b. On average, the gastric pH of piglets fed FERM_45 was lower (P<0.05) than for piglets fed FERM_0. On average, there was a tendency (P<0.10) for higher pH in SI1 of piglets fed FERM_45. The pH in SI2 and SI3 was not affected by diet.

On average over d4 and d8, the total content of SCFA was higher (P<0.01) in the stomach of piglets fed FERM_45 than for piglets fed FERM_0, whereas in SI1, SI2 and SI3 no dietary effect was observed. Gastric lactic acid content was about three-fold higher for piglets fed FERM-45 than for piglets fed FERM_0 (P<0.001). On average over d4 and d8, the lactic acid content in SI2 was higher for piglets fed FERM_45 (P<0.05) than for piglets fed FERM_0. Except for a higher propionic content in the stomach of piglets fed FERM_45 compared with piglets fed FERM_0 (P<0.10), no effect of dietary treatment on contents of propionic acid, acetic acid and butyric acid were observed.

Morphology Characteristics

The morphological characteristics are given in Table 5. In SI1, the effects of dietary treatment on morphological characteristics were obvious, whereas in SI2 and SI3 no effects of dietary treatment were observed. On average over d4 and d8, villous length in SI1 was higher for FERM_45 than for FERM_0 (P<0.01). In SI1, the villous to crypt ratio was higher for FERM_45 than for FERM_0 (P<0.001). In addition, there was a tendency towards favorable villous shape in SI1 for FERM_45 compared with FERM_0 (P<0.10). In none of the segments an effect of dietary treatment for the number of goblet cells, ratio of sulfo goblet cells and mitosis index was observed.

Table 3. Microbiology (log_{10} cfu/ml digesta) in the stomach and small intestine (I, II and III) of weaned piglets dissected at day 0, day 4 or day 8 after weaning and fed diets with 0% (FERM_0) or 45% (FERM_45) fermented wheat

	Day 0		Day 4		Day 8			P-value	
	Average	sd	FERM_0	FERM_45	FERM_0	FERM_45	SEM	Day	Diet
Stomach									
Lactic acid bacteria	7.9	0.32	7.5	9.8	7.4	9.6	0.11	0.252	<0.001
Yeasts	4.5	0.19	5.4	6.9	5.7	6.3	0.25	0.556	0.024
Total Coliforms	7.7	0.17	6.2	7.0	7.3	6.5	0.60	0.626	0.975
E.Coli	5.6	0.18	4.2	5.5	5.1	5.7	0.64	0.485	0.224
Small intestine I[1]									
Small intestine II									
Lactic acid bacteria	8.0	0.71	8.1	9.8	7.3	9.4	0.42	0.286	0.020
Yeasts	5.5	0.60	5.9	7.7	6.2	7.3	0.28	0.783	0.014
Total Coliforms	7.7	0.94	6.7	7.7	8.5	8.1	0.60	0.169	0.634
E.Coli	6.4	0.58	5.3	6.7	6.6	6.6	0.43	0.248	0.197
Small intestine III									
Lactic acid bacteria	6.8	0.24	8.0	9.3	7.6	8.4	0.52	0.320	0.145
Yeasts	5.8	0.10	6.5	7.3	7.0	7.1	0.21	0.400	0.126
Total Coliforms	8.8	0.51	7.7	8.6	9.3	9.2	0.52	0.147	0.561
E.Coli	6.5	0.21	6.1	6.8	7.5	7.4	0.41	0.108	0.552

[1] In this part not enough chymus could be taken for reliable microbial analyses.

Table 4a. pH and contents of organic acids (mmol/l digesta) in the stomach and small intestine (I, II and III) of weaned piglets dissected at day 0, day 4 or day 8 after weaning and fed diet with 0% (FERM_0) or 45% (FERM_45) fermented wheat

	Day 0		Day 4		Day 8			P-value	
	Average	sd	FERM_0	FERM_45	FERM_0	FERM_45	SEM	Day	Diet
Stomach									
PH	3.2	0.70	4.1	3.6	3.9	3.8	0.135	0.981	0.021
Total acids[1]	15.74	9.79	62.54	133.19	105.07	144.87	18.043	0.144	0.005
Lactic acid	8.64	7.64	24.53	86.22	23.18	67.85	14.981	0.516	0.001
Acetic acid	4.78	3.36	30.82	31.48	58.41	51.70	8.094	0.006	0.712
Propionic acid	0.51	1.29	3.28	8.83	10.83	14.88	2.384	0.008	0.054
Butyric acid	1.81	2.74	3.92	6.66	12.66	10.45	2.497	0.018	0.915
Small intestine I									
PH	5.9	0.68	6.1	6.1	5.2	6.0	0.190	0.015	0.075
Total acids[1]	6.90	5.74	24.20	23.46	29.44	34.56	7.886	0.310	0.783
Lactic acid	3.17	3.13	9.52	13.81	12.40	19.25	4.579	0.372	0.235
Acetic acid	1.80	2.12	14.48	9.14	17.03	14.48	3.943	0.327	0.327
Propionic acid	0.14	0.34	0.20	0.50	0.00	0.83	0.446	0.879	0.213
Butyric acid	1.79	3.32	0.00	0.00	0.01	0.00	0.007	0.257	0.523

[1] Total short chain fatty acids = lactic acid + acetic acid + propionic acid + butyric acid.

Table 4b. pH and contents of organic acids (mmol/l digesta) in the small intestine (part II and III) of weaned piglets dissected at day 0, day 4 or day 8 after weaning and fed diet with 0% (FERM_0) or 45% (FERM_45) fermented wheat

	Day 0		Day 4		Day 8			P-value	
	Average	sd	FERM_0	FERM_45	FERM_0	FERM_45	SEM	Day	Diet
Small intestine II									
PH	5.7	0.38	5.9	6.0	6.0	5.9	0.118	0.798	0.929
Total acids[1]	30.15	35.75	22.71	28.68	40.36	45.70	8.759	0.058	0.524
Lactic acid	6.52	4.70	9.90	18.14	11.91	27.40	4.041	0.175	0.007
Acetic acid	5.05	6.70	12.79	10.29	27.51	18.30	5.953	0.066	0.334
Propionic acid	8.41	13.30	0.02	0.25	0.00	0.00	0.123	0.283	0.378
Butyric acid	10.17	19.55	0.00	0.00	0.94	0.00	0.472	0.326	0.326
Small intestine III									
PH	6.7	0.50	6.9	6.5	6.8	6.6	0.230	0.998	0.229
Total acids[1]	93.82	104.36	51.59	61.90	70.78	47.71	14.036	0.860	0.653
Lactic acid	23.71	36.10	23.90	35.23	31.57	26.00	11.004	0.944	0.795
Acetic acid	36.29	39.35	26.84	26.29	39.21	21.28	7.769	0.639	0.244
Propionic acid	7.64	16.25	0.85	0.39	0.00	0.43	0.513	0.438	0.973
Butyric acid	26.18	61.46	0.00	0.00	0.00	0.00	0.000		

[1] Total short chain fatty acids = lactic acid + acetic acid + propionic acid + butyric acid.

Table 5. Morphological characteristics in the three segments of the small intestine of weaned piglets dissected at day 0, day 4 or day 8 after weaning and fed diet with 0% (FERM_0) or 45% (FERM_45) fermented wheat

	Day 0		Day 4		Day 8			P-value	
	Average	sd	FERM_0	FERM_45	FERM_0	FERM_45	SEM	Day	Diet
Small intestine I									
Villous length (µm)	447	103	278	321	295	398	21.4	0.037	0.002
Crypt depth (µm)	193	22	236	223	275	260	9.3	<0.001	0.150
Villous:crypt ratio (µm:µm)	2.35	0.61	1.18	1.45	1.09	1.54	0.088	0.985	<0.001
Shape villous[1]	0.41	0.40	0.71	0.66	1.17	0.75	0.132	0.042	0.087
Goblet (n/100 µm crypt)	6.45	1.57	4.64	4.06	5.66	6.69	0.514	0.002	0.668
Index of mitosis (n/100 crypt)	0.49	0.34	0.32	0.31	0.31	0.54	0.084	0.197	0.188
Small intestine II									
Villous length (µm)	389	111	256	219	261	271	17.8	0.120	0.457
Crypt depth (µm)	214	13	210	226	242	231	11.4	0.110	0.810
Villous:crypt ratio (µm:µm)	1.83	0.54	1.23	0.99	1.10	1.18	0.089	0.756	0.365
Shape villous	0.86	0.47	0.82	1.07	1.10	0.80	0.128	0.854	0.961
Goblet (n/100 µm crypt)	6.16	1.43	6.27	5.88	6.58	7.41	0.512	0.083	0.670
Index of mitosis (n/100 crypt)	0.58	0.45	0.73	0.73	0.82	0.61	0.142	0.906	0.474
Small intestine III									
Villous length (µm)	273	83	200	164	223	252	16.5	0.002	0.833
Crypt depth (µm)	159	24	199	183	210	237	9.9	0.003	0.586
Villous:crypt ratio (µm:µm)	1.82	0.82	1.01	0.90	1.08	1.07	0.075	0.116	0.422
Shape villous	0.85	0.43	0.88	0.85	0.97	0.94	0.129	0.470	0.807
Goblet (n/100 µm crypt)	8.33	1.33	8.54	7.96	10.39	9.55	0.687	0.019	0.311
Index of mitosis (n/100 crypt)	0.56	0.41	0.74	0.63	0.57	0.60	0.088	0.685	0.286

1 Villous shape measured following the method of Mouwen (1972) as described by Van Leeuwen et al. (1995).

Growth performance

Piglets fed FERM_45 had an average daily gain of 18.8 and 67.2 g/d for the period d0-d4 and d0-d8, respectively (data not shown) and piglets fed FERM_0 had an average daily gain of -15.6 and +35.9 g/d, respectively. No differences in feed intake were observed.

DISCUSSION

During the 24-h fermentation of liquid wheat, levels of starch and sugar decreased to 0.898 and 0.180 from the initial level, respectively (data not shown). The levels of lactic acid, acetic acid and ethanol in liquid wheat increased from zero to 42.4, 5.3 and 10.9 g/kg DM respectively after 24-h fermentation, whereas pH decreased from 5.9 to 3.6 (data not shown). This is in line with Moran et al. (in prep.), who reported a pH-value of 3.7, and 55.5 g lactic acid/kg DM and 4.9 g acetic acid/kg DM in 24-h fermented liquid wheat. As a consequence of the addition of 45% fermented liquid wheat, the energy supply of FERM_45, compared with FERM_0, is partly shifted from starch and sugar into lactic acid, acetic acid and ethanol (Table 1). Apart from the differences in nutrient composition, the microbial composition was also different between FERM_0 and FERM_45. FERM_45 is characterized by a high number of lactic acid bacteria and yeasts, but also by a higher number of total Coliforms and *E.coli*. This seems to be caused mainly by the high number of total Coliforms and *E.Coli* in the 24-h fermented wheat (data not shown). This is unexpected, since the fermented wheat has a pH of 3.6, and it is generally considered that a low pH inhibits the growth of harmful bacteria, such as Coliforms (Nout et al., 1989). Our observation suggests that 24-h fermentation of wheat is not enough to eliminate Coliforms and that besides the absolute pH-value also the time aspect is important. This is confirmed by Moran et al. (in prep.), who showed that in 24-h fermented wheat the population of Coliforms was still high (5.13 $\log_{10}$ cfu/g; pH-value 3.7), whereas in 48-h fermented wheat the number of Coliforms was <3.0 $\log_{10}$ cfu/g.

The effect of feeding a partly fermented diet on pH-value and level of SCFA is most obvious in the stomach, whereas in the small intestine almost no difference was observed. The feeding of FERM_45 to weanling piglets resulted in a reduced gastric pH and higher levels of gastric lactic acid. So far, data on effects of fermented liquid diets on levels of SCFA in the chymus of weanling piglets is scarce. In agreement with the present study, lower gastric pH and higher gastric lactic acid content were found in piglets fed a completely fermented compound diet for 14 days (Mikkelsen and Jensen, 1997) and 28 days (Mikkelsen and Jensen, 1998) after weaning. In both experiments, the authors also observed a higher pH in the first part of the small

intestine when piglets were fed a completely fermented diet (Mikkelsen and Jensen, 1997; 1998). In the present study, however, only a numerically higher pH in SI1 was found for piglets fed FERM_45 compared with piglets fed FERM_0 (6.0 compared to 5.2 on D8). This might be due to several factors. In our trial the piglets were dissected at younger age compared to Mikkelsen and Jensen (1997, 1998), who used piglets fed fermented diets till 14 and 28 days after weaning. Because there is a positive age-effect on pancreatic secretion (Tarvid et al., 1994), the older piglets had higher pancreatic secretion to buffer pH in the small intestine. In addition, older pigs have higher daily feed intake. Combined with the fact that Mikkelsen and Jensen used completely fermented diets, whereas we used partly fermented diets, the daily intake of SCFA was higher. Consequently, it might be assumed that the triggering effect of lactic acid or SCFA on pancreatic secretion was at a higher level in the trials of Mikkelsen and Jensen (1997, 1998). Another possibility is the time-effect. Mikkelsen and Jensen (1997, 1998) dissected the piglets 3 hours after feeding, whereas in our trial it was 1.5 hour. The time for pancreatic secretion to influence pH of the digesta is therefore different in both trials. Ravindran and Kornegay (1993) reported that low gastric pH stimulates the secretion of pancreatic bicarbonate, which can buffer the pH in the first part of the small intestine. Thaela et al. (1998) reported that the addition of 2.5% lactic acid to weanling diets increased the volume and protein content of pancreatic secretion. Although, they found no increased bicarbonate level (Thaela et al., 1998). It can be hypothesized that completely fermented diets with levels of lactic acid up to 80 g/kg DM and levels of acetic acid up to 11 g/kg DM (Mikkelsen and Jensen, 1997), influence pancreatic secretion, including bicarbonate.

After weaning, villous shortening in the small intestine of piglets often occurs (Makkink, 1993; Van Beers-Schreurs, 1996). In literature mainly the effect of feed intake on mucosal morphology is studied, while in the present study the sort of nutrient is studied. The composition of FERM_45 was changed in such way that starch and sugars were partly replaced into the fermentation products lactic acid, acetic acid and ethanol. In the present study, it was clearly that the feeding of FERM_45 resulted in a smaller reduction (d4) and faster recovery (d8) of villous height compared with the feeding of FERM_0. The piglets fed FERM_45 showed higher lactic acid and total SCFA levels in the stomach, and higher villous height, better villous shape and higher villous to crypt ratio than piglets fed FERM_0. These findings, seem to be in agreement with findings of other studies in which positive effects of SCFA on intestinal proliferation (Sakata et al., 1995) or villous height (Nousiainen, 1991) were observed. Moreover, in the first part of the small intestine no significant differences in lactic acid and total SCFA levels were found between piglets fed FERM_45 or FERM_0. It might be suggested that SCFA are rapidly absorbed by the mucosal cells and used as a fuel, the so-called "luminal nutrition theory" (e.g., Pluske et al.,

1997).

The present study shows that the addition of a fermented carbohydrate rich feed ingredient to a liquid diet for weanling piglets changed the nutrient composition of the diet. The feeding of a diet with a high content of fermentation products had positive effects on the gastrointestinal characteristics of weanling piglets. The results clearly confirm that the type of nutrient is important for mucosal architecture and physiology of the chymus, mainly in the first part of the gastrointestinal tract. Fermentation is a complex process and fermented diets consists of a broad range of fermentation products (e.g. lactic acid, acetic acid, ethanol, microflora), and additional research is needed to verify the results found in the present study. Furthermore, it is interesting to get more knowledge about the responsible factor or combination of factors in fermented diets, which contribute to the observed positive effects on gastrointestinal characteristics. In addition, research on effects of addition of a fermented carbohydrate rich feed ingredient to a diet for weanling piglets on the average growth performance traits after weaning is needed.

IMPLICATIONS

This study has shown that the addition of a fermented carbohydrate rich feed ingredient (i.e., wheat) to a diet for weanling piglets, positively affects the physiology and villous architecture of first part of the gastrointestinal tract. Piglets fed the liquid diet with fermented wheat, had higher villous height and higher villous to crypt ratio. Also the villous shape was more favorable for the piglets fed the diet with the fermented wheat. Given the concept that short chain fatty acids might affect villous architecture directly after weaning, and consequently positively affect growth performance traits, the present data provide indications for a new feeding concept of feeding liquid diets with fermented carbohydrate rich feed ingredients to prevent undesirable changes in villous architecture of weanling piglets.

ACKNOWLEDGEMENTS

The assistance of Gisabeth Binnendijk, Hans Diepstraten, Nikolett Horváth, Piet van Leeuwen, Tamme Zandstra, Martin Rijnen and Melike Balk is gratefully acknowledged.

REFERENCES

CVB. 2000. Chemical composition, digestibility, and feeding value of feedstuffs. Veevoedertabel, Centraal Veevoederbureau, Lelystad, The Netherlands.

Jensen, B.B., Mikkelsen, L.L., 1998. Feeding liquid diets to pigs. In: P.C. Garnsworthy and J. Wiseman (ed.). Recent Advances in Animal Nutrition. pp 107-126. Nottingham University Press, Loughborough, England.

Kik, M.J.L., Huisman, J., Poel, A.F.B. van der, Mouwen, J.M.V.M., 1990. Pathologic changes of the small intestinal mucosa of pigs after feeding Phaseolus vulgaris beans. Vet. Pathol. 27, 329-334.

Nousiainen, J., 1991. Comparative observations on selected probiotics and olaquindox used as feed additives for piglets around weaning. 2. Effect on villous height and crypt depth in the jejunum, ileum, caecum and colon. J. Anim. Physiol. Anim. Nutr. 66, 224-230.

Makkink, C.A., 1993. Of piglets, dietary proteins and pancreatic proteases. Ph.D. dissertation. Wageningen Agricultural Univ., The Netherlands.

Mikkelsen, L.L., Jensen, B.B., 1997. Effect of fermented liquid feed (FLF) on growth performance and microbial activity in the gastrointestinal tract of weaned piglets. In: J.P. Laplace, C. Fevrier, and A. Barbeau (ed.). Digestive Physiology in Pigs. E.A.A.P. publication no. 88. pp. 639-642. 26-28 May, Saint Malo, France.

Mikkelsen, L.L., Jensen, B.B., 1998. Performance and microbial activity in the gastrointestinal tract of piglets fed fermented liquid feed at weaning. J. Anim. Feed Sci. 7, 211-215.

Mouwen, J.M.V.M. 1972. White scours in piglets at three weeks of age. Ph.D. dissertation, Univ. Utrecht, The Netherlands.

Nout, M.J.R., Rombouts, F.M., Havelaar, A., 1989. Effect of accelerated natural lactic fermentation of infant food ingredients on some pathogenic microorganisms. Int. J. Food Microbiol. 8, 351-361.

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.

Prescott, L.M., Harley, J.P., Klein, D.A., 1996. Microbiology. WCB Publishers, 935.

Ravindran, V., Kornegay, E.T, 1993. Acidification of weaner pig diets: a review. J. Sci. Food Agric. 62, 313-322.

Russell, P.J., Geary, T.M., Brooks, P.H., Campbell, A., 1996. Performance, water use and effluent output of weaner pigs fed ad libitum with either dry pellets or liquid feed and the role of microbial activity in the liquid feed. J. Sci. Food Agric. 72, 8-16.

Sakata, T., Adachi, M., Hashida, M., Sato, N., Kojima, T., 1995. Effect of n-butyric acid on epithelial cell proliferation of pig colonic mucosa in short-term culture. Dtsch. Tierärztl. Wschr. 102, 163-164.

Scholten, R.H.J., Peet-Schwering, C.M.C. van der, Verstegen, M.W.A., Hartog, L.A. den, Schrama, J.W., Vesseur, P.C., 1999. Fermented co-products and fermented compound diets for pigs: a review. Anim. Feed Sci. Technol. 82, 1-19.

Scholten, R.H.J., Rijnen, M.M.J.A., Schrama, J.W., Boer, H., Vesseur, P.C., Hartog, L.A. den, Peet-Schwering, C.M.C. van der, Verstegen, M.W.A., 2001a. Fermentation of liquid co-products and liquid compound diets: 1. Effects on chemical composition during a 6-day storage period. J. Anim. Physiol. Anim. Nutr. 85, 111-123.

Scholten, R.H.J., Rijnen, M.M.J.A., Schrama, J.W., Boer, H., Peet-Schwering, C.M.C. van der, Hartog, L.A. den, Vesseur, P.C., Verstegen, M.W.A., 2001b. Fermentation of liquid co-products and liquid compound diets: 2. Effects on pH, acid-binding capacity, organic acids and ethanol during a 6-day storage period. J. Anim. Physiol. Anim. Nutr. 85, 124-134.

Tarvid, I., Cranwell, L.M., Vavala, R., 1994. The early postnatal development of protein digestion in pigs. 1. Pancreatic enzymes. In: 6th international symposium on digestive physiology in pigs. Bad Doberan, Germany, p. 199-202.

Thaela, M.-J., Jensen, M.S., Pierzynowski, S.G., Jakob, S., Jensen, B.B., 1998. Effect of lactic acid supplementation on pancreatic secretion in pigs after weaning. J. Anim. Feed Sci. 7, 181-183.

Van Beers-Schreurs, H.G.M. 1996. The changes in the function of the large intestine of weaned pigs. Ph.D. dissertation. Univ. Utrecht, The Netherlands.

Van Leeuwen, P., Jansman, A.J.M., Wiebenga, J., Koninkx, J.F.J.G., Mouwen, J.M.V.M., 1995. Dietary effects of faba-bean (Vicia faba L.) tannins on the morphology and function of the small-intestinal mucosa of weaned pigs. Br. J. Nutr. 73, 31-39.

Chapter 6

LIQUID DIETS FOR WEANLING PIGLETS: EFFECTS OF FERMENTED WHEAT ON GROWTH PERFORMANCE AND HEALTH

Ronald H.J. Scholten[1,*], Carola M.C. van der Peet-Schwering[1], Johan W. Schrama[2], Leo A. den Hartog[1], Martin W.A. Verstegen[3]

[1] Research Institute for Animal Husbandry, Lelystad, The Netherlands

[2] Wageningen Institute of Animal Sciences, Fish Culture and Fisheries Group, Wageningen, The Netherlands

[3] Wageningen Institute of Animal Sciences, Animal Nutrition Group, Wageningen, The Netherlands

[*] Present address: Beuker Vochtrijke Diervoeders B.V., Doetinchem, The Netherlands

To be submitted to:

Livestock Production Science

ABSTRACT

The dose-response effects of adding fermented liquid wheat to diets for weanling piglets, on daily gain, feed to gain ratio, feed intake and health was studied. Fifty-two pens of four 28-old weanling piglets were each fed one of the four experimental diets, which were similar in composition except for the content of fermented liquid wheat. To ferment wheat water of 30 °C was added to milled wheat, in a 1:2.2 ratio. Afterwards, wheat was fermented by naturally present microbes for 24 h using a residue of 20% fermented wheat for a previous batch as inoculum. Non-fermented wheat was exchanged for different doses of fermented liquid wheat (**FW**). Diets contained 0, 15, 30, or 45% FW. Piglets were given access to liquid feed twice per day. Feeding level was calculated based on metabolic bodyweight of the piglets. Piglets were weighed at day of weaning (d0), d8, d13, d20, d27 and d34 after weaning. The 24-h fermentation of wheat resulted in lower pH and lower contents of starch and sugar, whereas contents of lactic acid, acetic acid and ethanol increased. The pH-value of the experimental diets was reduced by increased addition of FW from 6.0 to 4.7. The calculated daily gross energy intake was lowest for the piglets fed the diet with 45% FW. Piglets fed FW had numerical higher daily gain (+15 to +17 g/d; $P=0.135$) and significant better feed to gain ratio ($P<0.001$) compared with the control diet. No dose-response effects of FW on growth performance traits were observed. The present study indicates that the addition of a fermented carbohydrate-rich feed ingredient to a liquid diet for weanling piglets had positive effect on growth performance traits.

INTRODUCTION

Compared with feeding non-fermented liquid diets, the feeding of fermented liquid compound diets to weanling piglets increase daily gain and feed intake (Russell et al., 1996; Mikkelsen and Jensen, 1997). However, most trials focused on *ad libitum* feeding of fermented liquid compound diets showed that feed to gain ratio was mostly effected negatively (Mikkelsen and Jensen, 1997 and 1998; Ward et al., 2001; Pedersen, 2001). During fermentation of feedstuffs, levels of starch and sugar decrease, whereas levels of short chain fatty acids (**SCFA**) and ethanol increased (Scholten et al., 2001a and b). Fermentation decreases the pH of the liquid diets to values between 3.5 and 4.0 (Scholten et al., 2001b). This pH may be beneficial for pig health (Scholten et al., 1999; Partanen and Mroz, 1999). Pedersen (1999) reported loss of feeding value because about 25% of added synthetic lysine was transformed into other products during fermentation of liquid compound diets for 24 hours. In addition, undesirable products may be made during fermentation like ammonia and toxic amines, which can be harmful for the

gastrointestinal mucosa (Visek, 1972 and 1978). A fermentation concept which maintains the positive effects on daily gain and pig health, but prevent the risk of protein fermentation, and which is also able to improve feed to gain ratio, would be more desirable than the current practice of fermenting complete compound diets.

In practice, pigs fed a liquid diet with fermented carbohydrate rich products had higher daily gain and better feed to gain ratio compared to the pigs fed a non-fermented liquid diet (Scholten et al., 1999). Recently, Scholten et al. (2001c) showed that the inclusion of 45% fermented wheat to a liquid diet for weanling piglets had positive effects on gastrointestinal characteristics, e.g. villous height. Apart from these studies, however, little is known about the effects of adding fermented carbohydrate rich feedstuffs to pig diets on growth performance and health. It is unknown which proportion of a fermented feedstuff has to be added to a liquid diet to improve growth performance and health of piglets. Because weanling piglets are most susceptible for undesirable effects on growth performance and pig health directly after weaning, it is interesting to examine the effects of the addition of a fermented carbohydrate rich feedstuff to diets for weanling piglets. The aim of the present study was to determine the dose response effects of fermented liquid wheat on daily gain, feed to gain ratio and health of weanling piglets.

MATERIAL AND METHODS

Experimental design

In three trials of 35 days each, four dietary treatments were tested. For each dietary treatment, 52 piglets, housed in pens of four, were used. The experimental unit was a pen. Within trials, piglets were assigned to pens according to initial body weight (block design). Each block consisted of four pens and within a block the pens were allocated ad random to one of the four experimental diets (Table 1).

Animals

Two hundred and eight weanling piglets with an average age of 28 ± 0.1 days and an average body weight of 8.1 ± 0.1 kg (average ± SEM) were used. The piglets were derived from Dutch Landrace, Finnish Landrace, and Dutch Large White rotational-bred sow and Great Yorkshire terminal boars. During the suckling period all piglets had free access to water and creep feed was provided from day 10 after birth. On the day of weaning, sows were removed from the piglets at about 0900. Afterwards, the piglets were transferred to the nursery room. The day of weaning is defined as day 0 (d0).

Housing

Four piglets (two castrated males, two females) were housed per pen of 2.65 by 0.75 m. The pens had fully slatted floors that consisted of 1.8 m plastic slats and 0.85 m metal tri-bar slats. The pens were situated in a room with computer-controlled heating and mechanical ventilation systems. Room temperature was 28 °C at the start of the rearing period, and reduced gradually to a room temperature of 21 °C at the end of the rearing period. All pens were equipped with a trough in the front of the pen (0.25 m by 0.75 m), divided in 4 eating places. Piglets were given *ad libitum* access to water, which was supplied via nipples at the back of the pen. The care and treatment of the piglets were according to Dutch animal welfare legislation.

Diets and preparation of the diets

All diets had similar feedstuff composition (Table 1), except for the amount of non-fermented and fermented wheat. Non-fermented wheat (**NFW**) was exchanged for fermented wheat (**FW**), based on the weight after 24-h of fermentation. The four experimental diets contained:

FERM_0: 100% compound feed (including 45% NFW) + 0% FW;

FERM_15: 85% compound feed (including 30% NFW) + 15% FW;

FERM_30: 70% compound feed (including 15% NFW) + 30% FW;

FERM_45: 55% compound feed (without 0% NFW) + 45% FW.

In addition, piglets were fed a diet without antibiotics, without added organic acids, and without pharmacological levels of copper and zinc (Table 1).

Both the hammer milled wheat and the four types of compound feed (meal) were produced in one batch in amounts sufficient for the whole experiment, and stored at 4 °C during the experiment. The amount of feed needed for one trial was taken out of the cooling house one week prior to the start of the trial. During the trial the feed was stored in the temperature-controlled room (24 ± 1 °C).

Based on the method used in a preliminary study (Moran et al., in prep), FW was prepared by adding water of 30 °C in a water to feed ratio of 2.2:1 to hammer milled wheat (milled on a 3 mm sieve) about 24 hours before feeding. Two fermentation vessels were used; one for the morning feeding and one for the afternoon feeding. Every day, directly after the preparation of the diets, fresh dry wheat and water of 30 °C (ratio 1:2.2) was added to a residue of about 20% FW which remained as inoculum in the fermentation vessel. After the addition of fresh wheat and water, the liquid wheat was stirred continuously for five minutes, followed by stirring automatically every two hours for one minute. The two fermentation vessels were lockable, and situated in a temperature-controlled room (24 ± 1 °C).

Table 1. Composition of experimental diets as fed (g/kg)

	FERM_0	FERM_15	FERM_30	FERM_45
Ingredient, g/kg				
Wheat				
Non-fermented wheat	450	300	150	0
Fermented wheat	0	150	300	450
Barley	294	294	294	294
Wheypowder delactosed	62	62	62	62
Soya beans, extracted, hypro	50	50	50	50
Sunflower seed, extracted	30	30	30	30
Lineseed expeller	40	40	40	40
Fish meal	34	34	34	34
Potato protein	10	10	10	10
Fat	7.5	7.5	7.5	7.5
Limestone	7.1	7.1	7.1	7.1
Monocalciumphosphate	1.5	1.5	1.5	1.5
Lysine	6.1	6.1	6.1	6.1
Methionine	0.8	0.8	0.8	0.8
Threonine	0.8	0.8	0.8	0.8
Salt	1.0	1.0	1.0	1.0
Phytase	0.2	0.2	0.2	0.2
Vitamin and mineral premix[1]	5.0	5.0	5.0	5.0
Net Energy calculated (MJ/kg)[2]	9.68	9.68	9.68	9.68

[1] Provided the following amount of vitamins and minerals per kilogram of complete diet (88% dry matter): vitamin A, 7.000 IU; vitamin D3, 1.400 IU; vitamin E, 12 mg; vitamin B2, 4 mg; panthotenic acid, 7 mg; niacin, 18 mg; biotin, 0.1 mg; folic acid, 1.5 mg; choline chloride, 250 mg; Fe as FeSO4·7H2O, 100 mg; Cu as CuSO4·5H2O, 15 mg; Zn as ZnSO4·H2O, 68 mg; Co as CoSo4·7H2O, 0.25 mg; Mn as MnO2, 24 mg; Se as Na2SeO3·5H2O.

[2] Calculation based on net energy formula of CVB (2001).

Diet FERM_0, the control diet, was prepared about one hour before feeding, whereas diets FERM_15, FERM_30 and FERM_45 were prepared about 45 minutes before feeding. The supplementary feed was mixed with water of 30 °C (2.2:1), followed by the addition of FW. Components of the final diets (water, FW, supplementary compound feed, control feed) were weighed separately on a weight-scale, put into a clean, dry bucket and stirred for one minute. The buckets were closed and transported to the room with piglets. Just before feeding, the diet was stirred again and put into the feeding through. After feeding, all buckets were cleaned with

water and dried.

Feeding of the piglets

Piglets were fed restrictively twice a day (800 and 1600) and received the same diet continuously from d0 (day of weaning) till d34 after weaning. Piglets were given *ad libitum* access to drinking water. To prevent fermentation of the liquid feed in the through, feed refusals were removed 1.5 hours after the start of feeding during the first week after weaning. During the remainder of the rearing period, feed refusals were removed about 1 hour before the start of the next feeding time. The total amount of feed offered and the feed removals were recorded daily.

To exclude possible effects of feed intake on daily gain and feed to gain ratio, all piglets were fed the same amount of feed. The feeding level was based on the metabolic weight of the piglets. Piglets were weighed individually at d0, d8, d13, d20, d27 and d34 after weaning. The time of weighing was between 0900 and 1000. After every weighing the average metabolic body weight per pen was calculated. Based on daily gain data of several previous experiments carried out at our research institute, the assumed daily gain was 150, 250, 400, 500 and 600 g/day for the periods d0-d8, d9-d13, d14-d20, d21-d27, d28-d34, respectively. The assumed energy requirements for maintenance was arbitrary assumed at 500 KJ ME/kg $^{0.75}$. The energy content of all diets was 9.68 KJ NE/kg, as calculated by the currently used energy formula in the Dutch feed evaluation system for pigs (CVB, 2000), what is equal to 13.8 MJ ME/kg. Based on present data of energy intakes in previous experiments with weaned piglets carried out at our institute, we decided to start at d0 with a feeding level of 0.7 times the energy requirements for maintenance. This level was increased daily with 0.1 up to a maximum of 3.5 times the energy requirements for maintenance, which was reached at d28.

Feed sampling and analytical procedures

The pH of the fermented wheat and the four liquid diets were measured (CONSORT, Belgium) at each feeding time. Before measuring pH-value, the individual diets were stirred.

During the trial, weekly samples of dry wheat and the four types dry (supplementary) compound diets were taken to obtain one mixed sample per feed type. After each trial, the samples were stored at -20°C. In addition, FW was sampled twice per trial, and directly stored at –20 °C. Dry wheat, FW and dry compound diets were analyses for dry matter (DM), crude protein (CP), crude ash (ASH), crude fibre (CF), crude fat (CFAT), starch, sugars and amino acids (lysine, methionine, cystine, threonine, tryptophan). In addition, FW and the four final liquid diets were analysed for ethanol, lactic acid and acetic acid.

Furthermore, the weight change of the wheat in the fermentation vessels was measured during the experiment. From in total 13 different days the initial weight and the weight after 24 hours of fermentation was measured to quantify (weight) losses during fermentation.

All analyses were carried out in duplicate, except for the dry matter analyses, which were carried out in triplicate. Starch concentration was determined according to NIKO-MEMO 93-302, as described by Goelema et al. (1998); sugars were determined by HPLC according to Rocklin and Pohl (1983). CFAT, DM, ASH and N were determined according to the standard methods ISO/DIS 6492 (ISO, 1996), ISO 6469 (ISO, 1983), ISO 5984 (ISO, 1978) and ISO 5983 (ISO, 1979), respectively. Multiplying the nitrogen content with 6.25 calculated CP. Samples used for determination of acetic acid were centrifuged (41.750 G; 4°C) and the supernatant was extracted and diluted with phosphoric acid in a ratio 20:1. In this dilution, acetic acid was analysed by Gas Chromatography (GC) (Williams et al., 2000). Samples used for determination of lactic acid and ethanol were centrifuged (41.750 G; 4°C) and the supernatant was extracted and a few drops sulphuric acid were added to reach pH 2.0. In this supernatant, lactic acid and ethanol were analysed by High Performance Liquid Chromatography (HPLC) (Ehrlich et al., 1981).

Growth performance and pig health

The piglets were weighed individually at d0, d8, d13, d20, d27 and d34 after weaning. Feed amount and feed refusals were recorded at each feeding time (kg liquid diet). The body weight and the feed intake were used to calculate daily weight gain, daily feed intake, and feed to gain ratio (kg feed intake / kg weight gain). The data of feed intake and feed to gain ratio were calculated per pen. Feed intake per day was calculated as feed offered minus feed refusal. Feed intake was recalculated to 1) compound feed equivalents (88% dry matter) and 2) 100% dry matter. This recalculation was based on the dry matter of the diets, as analysed. The total daily nutrient intake was calculated, based on the average daily feed intake and the average analysed chemical composition of the diets.

During weighing of the piglets, the consistency of the faeces was determined visually for each piglet. Three types of consistency were distinguished: normal, soft and watery. For every weighing period, the proportion of consistency types of the faeces was calculated per treatment. Veterinary treatments of the piglets were recorded. When a piglet died, the (assumed) reason, date and weight were recorded. In total three piglets were taken out of the experiment and not taken into account in the results.

Statistical analyses

All statistical procedures were carried out using the General Linear Model procedure of SAS (SAS Inst. Inc., Cary, NC). Daily gain, feed intake, feed to gain ratio, energy intake and energy conversion ratio were analysed using the model given by Equation 1, in which the pen was used as the experimental unit (n=52). Preliminary analyses showed that for none of the measured parameters a significant dose-response effect was present. Therefore, the used model was:

$Y_i = \mu + T_i + BL_{(i)j} + D_k + e_{ijk}$ [1]

where Y_i = dependent variable; μ = overall mean; T_i = trial (i=1,2,3); $BL_{(i)j}$ = block effect within trial; D_k = diet (i = 1, .., 4); e_{ijk} = error term.

RESULTS

Fermentation of wheat

The nutrients of non-fermented liquid wheat (**NFW**) and fermented liquid wheat (**FW**) are given in Table 2.

At the start of the fermentation the pH-value of NFW was 5.9, whereas after 24 hours of fermentation the pH-value was decreased to 3.6. No differences in crude protein, crude fibre and ash were observed between NFW and FW. In contrast, after 24-hours fermentation, the level of starch and sugars decreased to 0.90 and 0.18 of the initial value. The levels of lactic acid, acetic acid and ethanol increased during the 24-h fermentation period. During 24-h of fermentation of wheat the levels of lysine (+7.9%), cystine (+4.4%) and threonine (+5.1%) increased.

During the 24-h fermentation of liquid wheat, the weight change was minimal. The weight of the wheat after 24-h fermentation was 0.994 of the initial weight at the start of the fermentation (data not shown). Based on the GE energy calculation, the energy value of liquid wheat decreased from 15.53 to 15.09 MJ GE/kg during the 24-h fermentation period.

Table 2. Average of the analysed pH and chemical composition of non-fermented wheat (NFW) and 24-hour fermented wheat (FW)

	NFW	FW
PH[1]	5.9	3.6
Nutrients (g/kg DM)[2]		
Crude Protein	133.3	128.3
Crude Ash	18.5	18.3
Crude Fibre	24.4	22.5
Crude Fat	24.0	21.3
Starch	641.9	576.6
Sugars	12.5	2.3
Lysine	3.05	3.29
Methionine	1.96	1.92
Cystine	2.71	2.83
Threonine	3.34	3.51
Tryptophane	1.45	1.42
Lactic acid	n.d.[3]	42.4
Acetic acid	n.d.	5.3
Ethanol	n.d.	10.9
Energy Value (MJ GE)[4]	15.53	15.09

[1] PH as measured in product: NFW n = 10 samples, FW n = 100 samples.
[2] Nutrients based on three samples (NFW) and six samples (FW).
[3] Not detected in fresh wheat, values assumed to be zero.
[4] Energy prediction based on gross energy content by multiplying the total intake of nutrients by the heat of combustion (kJ/g). Combustion heat: CP 23.6 kJ/g, CFAT 39.6 kJ/g, starch 17.5 kJ/g, sugar 15.7 kJ/g, lactic acid 15.2 kJ/g, acetic acid 14.6 kJ/g and ethanol 29.8 kJ/g (WEAST, 1968).

Composition of the diets

The pH-values at feeding were 6.0, 5.3, 4.9 and 4.7 for FERM_0, FERM_15, FERM_30 and FERM_45, respectively (Table 3). The total daily intakes of dry matter, crude protein, crude ash and crude fat were similar for the four diets (Table 3). The intake of starch, expressed as ratio compared to FERM_0, was 0.97, 0.98 and 0.93 for FERM_15, FERM_30 and FERM_45, respectively. The ratios for sugar were 0.88, 0.75 and 0.63 for FERM_15, FERM_30 and FERM_45, respectively, compared with FERM_0. The intake of nutrients in the groups FERM_15, FERM_30 and FERM_45 were thus shifted from starch and sugars to lactic acid, acetic acid and ethanol (Table 3).

Table 3. pH and the average daily intake of nutrients (g) of the piglets fed diets with 0% (FERM_0), 15% (FERM_15), 30% (FERM_30) and 45% (FERM_45) fermented wheat

	DIET			
	FERM_0	FERM_15	FERM_30	FERM_45
pH[1]	6.0	5.3	4.9	4.7
Average daily intake per pig (g)[2]				
Dry Matter	455	451	463	455
Crude Protein	86	87	86	86
Crude Fibre	22	18	18	18
Crude Ash	24	24	24	24
Crude Fat	22	22	22	22
Starch	213	206	208	198
Sugars	24	21	18	15
Lysine	4.2	4.5	4.4	4.4
Methionine	2.0	1.8	1.7	1.8
Cystine	1.4	1.4	1.4	1.4
Threonine	3.3	3.1	3.1	3.1
Tryptophan	0.9	0.9	0.9	0.9
Lactic acid	2.8	6.6	9.1	12.3
Acetic acid	0.6	1.2	1.6	2.0
Ethanol	0.4	1.0	2.1	2.5
Predicted Energy Intake[3] (MJ GE)	7.07	7.01	7.05	6.89

[1] PH measured in the diet as fed.
[2] Per diet n=3 samples were analysed. In combination with the average daily feed intake (based on average DM intake of whole rearing period) the average daily nutrient intake was calculated.
[3] Energy prediction based on gross energy content by multiplying the total intake of nutrients by the heat of combustion (kJ/g). Combustion heat: CP 23.6 kJ/g, CFAT 39.6 kJ/g, starch 17.5 kJ/g, sugar 15.7 kJ/g, lactic acid 15.2 kJ/g, acetic acid 14.6 kJ/g and ethanol 29.8 kJ/g (WEAST, 1968).

Growth performance

The average growth performance traits are given in table 4. In the first 13 days after weaning, no differences in daily gain, daily feed intake and feed to gain ratio were observed (P>0.10). From day 14 till day 34, there was a numerically higher daily gain (P=0.105) and a significant lower feed to gain ratio (P<0.01) when 15%, 30% or 45% FW was added to the diet. Between the three diets with FW, no differences in daily gain and feed to gain ratio were observed.

Table 4. Growth performance results of weaned piglets fed diet with 0% (FERM_0), 15% (FERM_15), 30% (FERM_30) or 45% (FERM_45) fermented wheat during 5 weeks after weaning

	FERM_0	FERM_15	FERM_30	FERM_45	SEM	P
Number of piglets	51	52	51	51		
Number of pens	13	13	13	13		
Period d0-d13						
Initial weight (kg)	8.1	8.1	8.1	8.1		
Daily gain (g)	125	128	131	133	5.0	0.699
Feed intake (g/day)	209	211	213	213	2.9	0.721
Feed intake (g DM/day)	184	185	187	188	2.6	0.721
Feed to gain ratio	1.74	1.69	1.67	1.63	0.06	0.612
Feed to gain ratio (DM)[1]	1.53	1.49	1.47	1.43	0.05	0.612
Period d14-d34						
Initial weight d14 (kg)	9.8	9.8	9.9	9.9		
Daily gain (g)	375	398	400	399	8.0	0.105
Feed intake (g/day)	708	700	720	705	11.5	0.631
Feed intake (g DM/day)	623	616	634	621	10.1	0.631
Feed to gain ratio	1.90[a]	1.76[b]	1.81[b]	1.78[b]	0.02	0.001
Feed to gain ratio (DM)[1]	1.67[a]	1.55[b]	1.59[b]	1.57[b]	0.02	0.001
Period d0-d34						
Daily gain (g)	280	295	297	297	6.0	0.135
Feed intake (g/day)	517	513	526	517	8.0	0.683
Feed intake (g DM/day)	455	451	463	455	7.2	0.683
Feed to gain ratio	1.86[a]	1.74[b]	1.78[b]	1.76[b]	0.02	0.001
Feed to gain ratio (DM)[1]	1.64[a]	1.53[b]	1.57[b]	1.54[b]	0.02	0.001

[a,b] Different superscript in row means significant difference $P<0.05$.
[1] Calculated as (dry matter intake in kg / weight increase in kg).

Veterinary treatments and faeces viscosity

During the experiment in total three piglets died, divided over three treatments. Between the dietary treatments no difference in the total number and the type of veterinary treatments was observed (data not shown). No differences in faeces viscosity between the dietary treatments as well as between the five individual weeks of the rearing period were observed (data not shown).

DISCUSSION

General

The aim of this study was to determine the dose response effects of fermented liquid wheat on daily gain, feed to gain ratio and health in weanling piglets. The piglets were fed restrictively to prevent differences in feed intake, which may be confounding with growth performance traits. The consequence of this restricted feeding level, is a lower average daily feed intake and average daily gain compared to experiments carried out with the same genotype of piglets and housing conditions, but with *ad libitum* access to feed (Bruininx et al., 2001).

Fermentation process

Since sugars and starch are sources for fermentation, their levels decreased during 24-h of fermentation of wheat. On average, after 24-h of fermentation of wheat, the level of starch and sugar was 0.90 and 0.18 from the initial level at the start of the fermentation. Per kg DM, about 75 g starch + sugars was transformed into fermentation products, like SCFA, ethanol and CO_2. From Table 2 it can be derived, that fermented wheat (FW) contained in total 48 g lactic acid + acetic acid /kg DM. Moran et al. (in prep) reported about 60 g lactic + acetic acid/kg DM in wheat that was fermented for 24-h. The proportion lactic acid to acetic acid was 89:11 in the current trial, which is in good agreement with the proportion of 92:8 found by Moran et al. (in prep). This proportion is an indication that the main microbial populations were lactic acid bacteria of the homo-fermentative type. Homo-fermentative lactic acid bacteria produce out of 1 unit glucose about 0.90 lactic acid, while the remaining 0.10 is acetic acid, ethanol and CO_2 (Prescott et al., 1996). The formation of ethanol, being 10.9 g/kg DM in the present study, is probably the result of the presence of yeasts and Coliforms (Prescott et al., 1996), which reached levels of 3.9 and 9.6 $\log_{10}$ cfu/mg, respectively, in the used FW (Scholten et al., 2001c).

Growth performance

As shown in Table 3, the experimental design to get comparable daily intake of dry matter between the four treatment groups and to shift the intake of energy from starch + sugars into fermentation products was successful.

Experiments that focused on feeding completely fermented compound diets to weanling piglets showed that feed to gain ratio was mostly effected negatively (Russell et al., 1996; Mikkelsen and Jensen, 1997 and 1998; Ward et al., 2001; Pedersen, 2001). In the current trial, the inclusion of 15%, 30% and 45% FW in a diet for weanling piglets resulted in an improvement of feed to gain ratio. On average, the improvement was between 4.3 and 6.4% compared to the

control diet, FERM_0. Our hypothesis that the addition of a fermented carbohydrate rich feedstuff will improve the feed to gain ratio is clearly demonstrated in the present trial. In another study, Scholten et al. (1999) reported a better feed to gain ratio (± 4.0%) when fermented carbohydrate rich feedstuffs were added to the liquid diet of growing and finishing pigs. However, more experiments about the effects of the addition of individually carbohydrate rich feedstuffs on performance traits, is not reported in literature. Additional research to verify the effect of this new fermentation concept on the growth performance traits is needed.

It is interesting to know why the addition of fermented carbohydrate rich feedstuffs improve feed to gain ratio, whereas fermentation of complete compound diets have a negative effect on feed to gain ratio. Pedersen (1999, 2001) reported that between 25 and 31% of the added synthetic lysine was lost during the fermentation of complete compound diets. This may be a possible contributing factor for the negative effects on the feed to gain ratio in weanling piglets in literature. In contrast, the level of lysine in the present study increased from 3.05 g to 3.29 (+7.8%; Table 2) during the 24-h of fermentation of the carbohydrate rich feedstuff liquid wheat. It seems that microbial population produced lysine during the fermentation. It cannot be excluded that in studies in which complete, protein-rich compound diets were fermented, crude protein was transformed by e.g. Coliforms into undesirable breakdown products. Coliforms are the main producers of toxic amines and ammonia (Dierick et al., 1986a and b), which are harmful for the gastrointestinal mucosa (Visek, 1972 and 1978). Moreover, in protein-rich compound diets, it takes relatively long time before the pH-value is reduced from approximately 6.0 to below 4.5 (Russell et al., 1996; Jensen and Mikkelsen, 1998). Below this pH, the number of Coliforms is reduced (Russell et al., 1996). During the high pH-phase, the presence of a high protein level and the high numbers of micro-organisms can contribute to the formation of protein breakdown products. More specific experimental data regarding the effects of fermentation of pig diets on changes of protein level, amino acid composition and levels of protein breakdown products is needed.

Surprisingly, in the present study no dose-response effects of the proportion of FW on growth performance traits were found. The addition of 15%, 30% or 45% FW seem to have similar effects. One possible explanation might be the restricted feed intake. In human food industry, lactic acid is used as a flavour enhancer (Shelef, 1994). From a limited number of experiments, it seems that the addition of lactic acid to pig diets increased feed intake in pigs (Scholten et al., 1999). Also Russell et al. (1996) and Jensen and Mikkelsen (1997) reported higher feed intakes when piglets were fed fermented diets instead of non-fermented diets. In our trial, however, the higher level of lactic acid in the diets with higher proportion of fermented wheat, could not affect the feed intake of the piglets because of the restricted feeding level. Another possible reason

might be that there is an optimum level of lactic acid, or total SCFA, to improve feed to gain ratio. A study of Roth et al. (1993) reported a positive effect of the addition of 16 or 24 g lactic acid / kg diet on feed to gain ratio, but they found no difference between the two levels. This is in line with results of Jongbloed and Jongbloed (1996) who observed no additional positive effect on feed to gain ratio when lactic acid level was increased from 16 to 32 g lactic acid/kg diet. In the current trial, the absolute lactic acid levels were about 6, 15, 20 en 27 g/kg DM for FERM_0, FERM_15, FERM_30 and FERM_45, respectively (data not shown). All these data indicates that, in the studied range from 6 to 32 g lactic acid/kg diet, there is no extra beneficial effect of lactic acid levels above 15 g/kg diet on feed to gain ratio.

Predicted Energy Value and Energy Intake

In the present study, the gross energy content at the start and at the end of the 24-h of fermentation of liquid wheat was calculated. This calculation was based on the specific values for the heat of combustion of all nutrients, including lactic acid, acetic acid and ethanol. We observed minor change in gross energy content during 24-h of fermentation. After 24-h of fermentation of wheat, the GE value was 97% of the initial GE value (Table 2). This loss of 3% of the GE value due to fermentation is similar to data of other fermented feedstuffs. Scholten et al. (2001c) reported GE losses between 1 and 3% after a six-day fermentation of several liquid co-products and liquid compound diets.

Based on the average daily nutrient intake in this experiment, the daily gross energy intake per piglet was calculated. Expressed as proportion to FERM_0, the average gross energy intake was 0.991, 0.997 and 0.975 for FERM_15, FERM_30 and FERM_45, respectively (Table 3). Despite the lower calculated GE intake, piglets fed FERM_45 showed a significant improved feed to gain ratio and a numerically higher daily gain. These effects can be caused by changes in availability of nutrients as a consequence of altered nutrients and / or their digestibility. Partanen and Mroz (1999) concluded that the addition of organic acids to pig diets had positive influence on the apparent total tract digestibility of crude protein and energy. This might also be true for fermented feedstuffs or diets. Knowledge about the effect of fermentation on the nutrient digestibility and availability, however, is very scarce. Gastrointestinal health may also be improved when fermented carbohydrate rich feedstuffs are fed to piglets. Scholten et al. (2001c) reported that weanling piglets fed a liquid diet with 45% fermented wheat had higher villous length, higher villous to crypt ratio and better villous shape compared to piglets with a liquid diet with 45% non-fermented wheat. This improved villous architecture may contribute to a higher absorption of nutrients from the lumen. Furthermore, maintenance processes may be altered due to fermentation products, as suggested by Schrama et al. (1997). Fermentation changed

the type of energy supply from carbohydrates into lactic acid, acetic acid and ethanol. Fermented diets contain organic acids and ethanol that are energy-yielding and which should be taken into account when producing pig diets. Nevertheless, even when the fermentation products are taken into account, as done in the gross energy calculation of this experiment, there is a discrepancy between the loss of gross energy and the improved growth performance. Therefore, there seem to be an additional energy-yielding or energy-saving effect of fermented feedstuffs, for which the mechanisms are still unclear.

ACKNOWLEDGEMENTS

The authors thank Gisabeth Binnendijk, Hans Diepstraten and Nikolett Horváth for their assistance in the experiment; Truus Post, Marianne van 't End, Huug Boer and Dick Bongers for carrying out chemical analyses; Martin Rijnen for critically reviewing the manuscript.

REFERENCES

Bruininx, E.M.A.M., Peet-Schwering, C.M.C. van der, Schrama, J.W., Vereijken, P.F.G., Vesseur, P.C., Everts, H., Hartog, L.A. den, and Beynen, A.C., 2001. Individually measured feed intake characteristics and growth performance of group-housed weanling pigs: Effects of sex, initial body weight, and body weight distribution within groups. J. Anim. Sci. 79, 301-308.

CVB, 2000. *Chemical composition, digestibility, and feeding value of feedstuffs.* Veevoedertabel, Centraal Veevoederbureau, Lelystad, The Netherlands.

Dierick, N.A., Vervaeke, I.J. Decuypere, J.A. and Henderickx, H.K., 1986a. Influence of the gut flora and of some growth-promoting feed additives on nitrogen metabolism in pigs. I. Studies in vitro. Livest. Prod. Sci. 14, 161-176.

Dierick, N.A., Vervaeke, I.J. Decuypere, J.A. and Henderickx, H.K., 1986b. Influence of the gut flora and of some growth-promoting feed additives on nitrogen metabolism in pigs. I. Studies in vivo. Livest. Prod. Sci. 14, 177-193.

Ehrlich, G.G., Goerlitz, D.,F., Bourell, J.H., Eisen, G.V. and Godsy, E.M., 1981. Liquid chromatography procedure for fermentation product analysis in the identification of anaerobic bacteria. App. Env. Microb. 42, 878-858.

Goelema, J.O., Spreeuwenberg, M.A.M., Hof, G., Poel, A.F.B. van der, Tamminga, S., 1998. Effect of pressure toasting on the rumen degradability and intestinal digestibility of whole and broken peas, lupins and faba beans and a mixture of these feedstuffs. Anim. Feed Sci. Technol. 76, 35-50.

ISO, 1978. Animal feeding stuffs – Determination of crude ash (ISO 5984). International Organization for Standardization, Genève, Switserland.

ISO, 1979. Animal feeding stuffs – Determination of nitrogen and calculation of crude protein content (ISO 5983). International Organization for Standardization, Genève, Switserland.

ISO, 1983. Animal feeding stuffs – Determination of moisture content (ISO 6496). International Organization for Standardization, Genève, Switserland.

ISO, 1996. Animal feeding stuffs – Determination of fat content, category B (ISO/DIS 6492). International Organization for Standardization, Genève, Switserland.

Jensen, B.B. and Mikkelsen, L.L., 1998. Feeding liquid diets to pigs. In: Recent Advances in Animal Nutrition (ed. P.C. Garnsworthy and J. Wiseman), pp. 107-126. Nottingham University Press, Loughborough, England.

Jongbloed, A.W., and Jongbloed, R., 1996. The effect of organic acids in diets for growing pigs on enhancement of microbial phytase efficacy. Report ID-DLO no. 96009. Lelystad, The Netherlands.

Mikkelsen, L.L., Jensen, B.B., 1997. Effect of fermented liquid feed (FLF) on growth performance and microbial activity in the gastrointestinal tract of weaned piglets. In: Digestive Physiology in Pigs (ed. J.P. Laplace, C. Fevrier, and A. Barbeau), E.A.A.P. publication no. 88. pp. 639-642. 26-28 May, Saint Malo, France.

Mikkelsen, L.L., Jensen, B.B., 1998. Performance and microbial activity in the gastrointestinal tract of piglets fed fermented liquid feed at weaning. J. Anim. Feed Sci. 7: 211-215.

Partanen, K., Mroz, Z., 1999. Organic acids for performance enhancement in pig diets. Nutrition Research Reviews 12, 1-30.

Pedersen, A. Ø., 1999. Styret fermentering af vådfoder. Kongres for Svineproducenter, 26-27 Oktober, Kopenhagen, Denmark.

Pedersen, A.Ø., 2001. Fermented liquid feed for weaners. Report no. 510, The National Committee for Pig Production, Danish Bacon and Meat Council, Denmark.

Prescott, L.M., Harley, J.P., Klein, D.A., 1996. Microbiology. WCB Publishers, London, 935 p.

Rocklin, R.D., Pohl, C.A., 1983. Determination of carbohydrates by anion exchange chromatography with pulsed amperometric detection. J. Liquid Chrom. 6, 1577.

Roth, F.X., Kirchgessner, M., Eidelsburger, U., 1993. Zur Nutritiven Wirksamkeit von Milchsäure in der Ferkelaufzucht. Agribiol. Res. 46, 229-239.

Russell, P.J., Geary T.M., Brooks, P.H., Campbell, A., 1996. Performance, water use and effluent output of weaner pigs fed ad libitum with either dry pellets or liquid feed and the role of microbial activity in the liquid feed. J. Sci. Food Agr. 72, 8-16.

Scholten, R.H.J., Peet-Schwering, C.M.C. van der, Verstegen, M.W.A., Hartog, L.A. den , Schrama, J.W., Vesseur, P.C., 1999. Fermented co-products and fermented compound diets for pigs: a review. Anim. Feed Sci. Technol. 82, 1-19.

Scholten, R.H.J., Rijnen, M.M.J.A., Schrama, J.W., Boer, H., Vesseur, P.C., Hartog, L.A. den, Peet-Schwering, C.M.C. van der, Verstegen, M.W.A., 2001a. Fermentation of liquid co-products and liquid compound diets: Part 1. Effects on chemical composition during a 6-day storage period. J. .Anim. Physiol. Anim. Nutr. 85, 111-123.

Scholten, R.H.J., Rijnen, M.M.J.A., Schrama, J.W., Boer, H., Peet-Schwering, C.M.C. van der, Hartog, L.A. den, Vesseur, P.C., Verstegen, M.W.A., 2001b. Fermentation of liquid co-products and liquid compound diets: Part 2. Effects on pH, acid-binding capacity, organic acids and ethanol during a 6-day storage period. J. Anim. Physiol. Anim. Nutr. 85, 124-134.

Scholten, R.H.J., Peet-Schwering, C.M.C. van der, Hartog, L.A. den, Schrama, J.W., Verstegen, M.W.A., 2001c. Fermented wheat in liquid diets: effects on gastrointestinal characteristics in weanling piglets. Sumbitted to J. Anim. Sci.

Schrama, J.W., Heetkamp, M.J.W., Bakker, G.C.M., Vught, P., Verstegen, M.W.A., 1997. Invloed van fermentatie van koolhydraten en vulling van maagdarmkanaal op de activiteit in relatie tot de energiestofwisseling bij vleesvarkens (in Dutch). Agricultural University Wageningen, The Netherlands.

Shelef, L.A., 1994. Antimicrobial effects of lactates: a review. J. Food Prot. 57, 445-450.

Visek, W.J., 1972. Effects of urea hydrolysis on cell life-span and metabolism. Proc. Fed American Soc. Exp. Biol. 31, 1178-1193.

Visek, W.J., 1978. The mode of growth promotion by antibiotics. J. Anim. Sci. 46, 1447-1469.

Ward, G.L., Moran, C.A., Miller, B.G., Brooks, P.H., Thorpe, J., 2001. Liquid feeding of newly weaned pigs: 1. The effects of feeding frequency and fermentation of liquid feed on growth performance and gastrointestinal morphology. In prep.

Williams, B.A., Oostdam, A.J., Groot, J.C.J., Boer, H., Tamminga, S., 2000: Effect of ageing of the in vitro fermentation of cell walls and cell contents of entire, fractionated, and composite leaves of Italian ryegrass. J. Sci. Food Agr. 80, 484-490.

GENERAL DISCUSSION

General Discussion

INTRODUCTION

Data from management programmes showed that pig farms using liquid co-products had favourable growth performance and health compared to pig farms not using liquid co-products (Scholten and Verdoes, 1997). At our research institute we found strong indications that the addition of liquid co-products to pig diets resulted in an increased daily gain, a better feed to gain ratio, less diarrhoea and a lower rate of mortality (Scholten et al., 1997; Van de Loo and Scholten, 1997). The reasons for these observations were not clear. Preliminary chemical analyses of the liquid co-products used in our experiments, showed that they contain high levels of the fermentation products lactic acid, acetic acid and ethanol. Our hypothesis was that fermentation might be involved in the observed improvements in performance and health when pigs were fed liquid co-products.

Fermentation is a dynamic process whereby carbohydrates and protein are transformed by microbes such as lactic acid bacteria (**LAB**), yeasts and Coliforms. Fermentation of carbohydrates yields mainly lactic acid, and to a lesser extent acetic acid, ethanol and CO_2. Fermentation of protein and amino acids is undesirable, due to the loss of feeding value and the production of ammonia and biogenic amines (e.g., cadaverine, histamine) that are harmful for the gastrointestinal mucosa (Visek, 1972, 1978). The fermentation process is influenced by many factors, like time, temperature, presence and type of substrate, and the presence and type of microbes. Knowledge about the fermentation kinetics and the effect on the chemical composition and feeding value of liquid fermented feedstuffs is lacking. Therefore, the effects of the fermentation process on the diet composition and energy value of several feedstuffs should be investigated.

It is generally considered that the addition of organic acids to dry compound diets lower gastric pH and acid binding capacity, and influence mucosal architecture (as reviewed by Partanen and Mroz, 1999). Therefore, fermented diets might influence gastrointestinal characteristics of weaned piglets. So far, however, hardly any attention has been given to the effect of fermented diets on gastrointestinal characteristics.

In the mid 90's research groups in England and Denmark started to study the effects of feeding fermented diets to piglets. They mainly focused on completely fermented compound diets (soaked with water), whereas hardly any attention has been given to the addition of individually fermented carbohydrate rich feedstuffs. In complete compound diets the risk of protein fermentation and amino acid breakdown is high (Pedersen, 1999, 2001). These protein breakdown products (e.g., ammonia, toxic amines) may be involved in the observed negative effect of fermented compound diets on feed to gain ratio (Russell et al., 1996; Jensen and Mikkelsen, 1998; Ward et al., 2001; Pedersen, 2001). A diet added with individually fermented carbohydrate rich feedstuffs might improve the performance of weanling piglets.

This thesis attempts to give more detailed insight into the effects of fermentation of liquid diets for pigs on 1) diet composition and energy value, 2) gastrointestinal characteristics, and 3) growth performance. In this thesis we studied commonly used feedstuffs in pig nutrition: three carbohydrate-rich liquid co-products (liquid wheat starch, mashed potato steam peels, cheese whey), two complete compound diets (liquid grower diet, liquid finisher diet) and one carbohydrate rich feedstuff (milled wheat).

EFFECTS ON DIET COMPOSITION

To achieve good growth performance it is necessary to have an accurate prediction of nutritional value of feedstuffs and diets. The feeding value depends on both the chemical composition and the digestibility coefficients of the nutrients. Knowledge on the chemical composition and digestibility of nutrients from most dry feedstuffs can be derived from feedstuff tables (e.g. CVB, 2001). From fermented feedstuffs (e.g. liquid co-products) data are scarce. In addition, an accurate prediction of nutritional value is more complex because of the effect of the fermentation process on chemical composition. In this thesis some experiments were carried out to study the effect of fermentation on the diet composition and change in feeding value of several feedstuffs and diets.

It is clearly shown that during fermentation the level of carbohydrates decreased whereas the level of fermentation products increased (Table 1). In general, the ratio of increase of fermentation products (g) : decrease of carbohydrates (g) was less than 1.00. This indicates there is an inefficiency in the transformation of carbohydrates into the fermentation products

lactic acid, acetic acid and ethanol. The formation of CO_2 may be one of the contributors to this inefficiency. In contrast to all individually fermented feedstuffs, the two liquid compound diets, in particular the liquid grower diet, had a higher output of fermentation products than the input of carbohydrates. Probably this may be due to an underestimation of the input of nutrients, because besides the carbohydrates also other nutrients like protein and non starch polysaccharides (NSP) may be transformed. However, besides our study, information about the changes in chemical composition of complete compound diets is scarce.

Table 1. Initial level of total carbohydrates (CH), change in total carbohydrates and the change in total fermentation products (TF) in liquid pig diets (g/kg)

	Storage Length (days)	Initial CH level Day 0	Change in		TF/CH	Ref.[3]
			CH[1]	TF[2]		
Liquid co-products[4]						
Liquid wheat starch	6	125.0	-7.5	3.3	0.44	A
Liquid wheat starch	21	111.3	-23.1	16.2	0.70	B
Liquid wheat starch	21	134.2	-15.1	11.3	0.75	B
Liquid wheat starch	21	112.8	-18.7	15.1	0.81	B
Potato steam peel	6	87.5	-17.2	14.4	0.84	A
Potato steam peel	21	39.6	-14.3	6.0	0.42	B
Potato steam peel	21	65.9	-10.9	4.3	0.40	B
Cheese whey	6	48.0	-13.0	9.1	0.70	A
Dry components[5]						
Liquid grower diet	6	114.2	-34.5	38.2	1.11	A
Liquid finisher diet	6	111.9	-33.5	34.0	1.02	A
Wheat	1	180.0	-28.4	15.4	0.55	C
Wheat	2	160.0	-29.8	13.1	0.44	D

[1] CH = total carbohydrates: starch + sugars.
[2] TF = *total fermentation products*: *lactic acid* + *acetic acid* + *ethanol*.
[3] References: A, Chapter 2+3; B, Smits (1998); C, Chapter 6; D, Chapter 4.
[4] Liquid co-products originating from human food industry.
[5] Dry feedstuffs or diets added with water to make a liquid mixture.

In addition to the duration of fermentation, the microbial population may be another reason for the observed variation between studies (Table 1). Lactic acid bacteria (**LAB**) and yeasts are the predominant microbe populations in fermented liquid diets (Russell et al., 1996; Mikkelsen and Jensen, 1997, 1998; Chapter 4 + 5). During fermentation the number of LAB

and yeast increase (Mikkelsen and Jensen, 1997, 1998; Chapter 4+5). Two types of LAB can be distinguished: homo-fermentative and hetero-fermentative. The first category produce out of 1 unit glucose about 0.9 unit lactic acid, whereas the second type produce out of 1 unit glucose 0.5 unit lactic acid and 0.5 unit acetic acid, ethanol and CO_2 (Prescott et al., 1996). Because the hetero-fermentative LAB produce also CO_2, these type may contribute to higher inefficiency of the transformation of carbohydrates into fermentation products (Table 1). The results in this thesis show also that the produced amount of ethanol is different between the studied feedstuffs. It is known that yeasts produce in anaerobic circumstances out of glucose about 50% ethanol and 50% CO_2, whereas in aerobic circumstances this is 100% CO_2 (Prescott et al., 1996). In all our experiments the storage vessels were locked and therefore considered to have anaerobic circumstances. The observed differences in levels of ethanol between the studied feedstuffs are probably due to differences in the number of yeasts. The high levels of ethanol in the complete compound diets and the liquid hammer milled wheat, maybe due to the naturally presence of yeasts on cereals. Another reason for the high level of ethanol in the complete compound diets may be the fact that during the first 48 hours of storage the pH was still above 4.5 (Chapter 3), and yeasts can multiply rapidly. Therefore, a quick drop in pH to below 4.5 to prevent high numbers of yeasts is necessary when feedstuffs or diets for pigs are fermented.

In addition to the duration of fermentation and the microbial population, temperature may also influence the proportion of fermentation products. Jensen and Mikkelsen (1998) clearly showed that increasing the temperature of the diet from 10 °C to 20 °C resulted in a three-fold higher level of lactic acid. Interestingly, the number of LAB was almost equal (about 10^9 cfu/g diet) and also the level of acetic acid was equal (Jensen and Mikkelsen, 1998). This suggests that temperature mainly affects the ratio of lactic acid to acetic acid. In the present thesis, the room temperature was kept constant (about 25 °C) and warm water (about 30 °C) was used to mix up the dry feedstuffs.

Several trials showed that if the duration of fermentation is long enough, the Coliforms present in the diet are eliminated (Russell et al., 1996; Jensen and Mikkelsen, 1997; Geary et al., 1998; Chapter 4). In contrast, in short-fermented diets the number of Coliforms remain at higher levels (Mikkelsen and Jensen, 1998; Chapter 4). Therefore, it is concluded that duration of fermentation is an important factor to eliminate potential harmful bacteria such as Coliforms.

It can be concluded that during the fermentation process, the chemical composition of pig diets is changed from starch and sugar into mainly lactic acid, and to a lesser extent acetic acid and ethanol. In general, on weight-base, the input of carbohydrates was higher than the output of fermentation products. By fermentation the microbial composition of the diet is moved into the direction of high numbers of LAB and to a lesser extent yeasts.

EFFECTS ON GASTROINTESTINAL CHARACTERISTICS AND ANIMAL HEALTH

Under practical conditions, positive effects of fermented liquid diets on reducing the rate of diarrhoea and mortality of pigs were reported by Pedersen et al. (1998) and Scholten et al. (1998). By fermentation the chemical and microbial composition of pig diets is changed, as described in the paragraph above. As hypothesized by Scholten et al. (1999; Chapter 1), fermented diets may positively influence the gastrointestinal characteristics.

Acidity of the GIT

Low gastric pH is essential for efficient digestion of proteins (pepsinogen activation) and to prevent the passage of potential harmful microbes such as Coliforms into the small intestine (reviewed by Partanen and Mroz, 1999). Furthermore, the end products of pepsin digestion and the low pH of digesta entering the duodenum are involved in the stimulation of pancreatic secretion, and they play a minor role in gastric emptying (reviewed by Partanen and Mroz, 1999). To obtain optimal enzymatic digestion in the small intestine, pH has to be increased by the addition of pancreatic bicarbonate.

The results found in the present study, regarding the pH-value in the GIT, showed that gastric pH was reduced and the pH in the first part of the small intestine showed a tendency for higher value (Chapter 5). This is in agreement with other studies focused on fermented diets (Jensen and Mikkelsen, 1997, 1998; Van Winsen et al., 2001a). Therefore, fermented diets may be used for influencing the pH in the stomach and first part of small intestine in a more desirable way.

The lower gastric pH may be the consequence of 1) the higher level of acids in the fermented diet and 2) a lower acid binding capacity of fermented diets (Chapter 2). Piglets fed a fermented liquid diet had higher lactic acid levels in the stomach and numerically higher levels in the small intestine compared with piglets fed a non-fermented liquid diet (Chapter 5). These findings are in line with results of Mikkelsen and Jensen (1997) and Van Winsen et

al. (2001a). Despite the higher acetic acid level in the fermented diet, piglets fed the fermented diet did not show higher levels of acetic acid in the stomach and small intestine (Chapter 5; Jensen and Mikkelsen, 1997). It may be suggested that acetic acid was absorbed rapidly. The absorption rate depends greatly upon pKa and the luminal pH (Partanen and Mroz, 1999). When luminal pH is below pKa value, SCFA are rapidly absorbed. The pKa values of acetic acid and lactic acid are 4.76 and 3.83, respectively. The luminal pH was between 3.6 and 3.8 (Chapter 5). Therefore acetic acid could be absorbed rapidly, whereas lactic acid remained present in the lumen. The piglets were dissected 1.5 hours after feeding. This indicates that the higher level of acetic acid consumed by piglets fed fermented diets, disappeared already 1.5 hour after feeding. Therefore, it might be suggested that the gastric pH-reducing effect of fermented diets high in acetic acid is less compared to fermented diets high in lactic acid.

A lower acid binding capacity of a diet means that in the stomach less HCL is needed to reduce the gastric pH to optimum levels for protein digestion. As a consequence the lower acid binding capacity of the diet also means that in the first part of the small intestine less bicarbonate is needed to increase pH to values necessary for optimum enzyme activity. Ravnindran and Kornegay (1993) reported that a low gastric pH stimulates the pancreas and bicarbonate secretion. This effect may be triggered by the high lactic acid content, as suggested by Thaela et al. (1998). This suggests that besides the lower acid binding capacity of fermented diets it self, there may be an indirect effect of the high level of lactic acid in fermented diets to the observed higher pH in the first part of the small intestine.

Microbial population in the GIT

Control of the activity and types of microbes in the gastrointestinal tract (**GIT**) is important to avoid undesired competition for nutrients between microbes and host, to restrict bacterial overgrowth (e.g., E. Coli, Salmonella) and to prevent production of harmful components (e.g. ammonia, biogenic amines). As described before, fermentation seem to influence the microbial composition of the diet it selves. LAB and yeast are dominant microbes, whereas Coliforms (Russell et al., 1996; Mikkelsen et al., 1997) and Salmonella (Van Winsen et al., 2001b) are less prevalent in fermented diets. As a consequence of the changed microbial diet composition, the microbial population in the GIT might be influenced as well. Jensen (1999) published data about the commonly present types of microbes in the GIT of pigs. In the stomach, lactic acid bacteria (**LAB**) are predominant, followed by Coliforms and yeasts. The number of yeasts is relative stable in the different segments of the GIT, whereas the

number of LAB and Coliforms increased towards the rectum (Jensen, 1999). In the current study, piglets fed fermented diet had higher numbers of LAB in stomach and small intestine than piglets fed the non-fermented diet (Chapter 5). This is in line with the results of Van Winsen et al. (2001a). It seems that the microbial population in the stomach interact with the microbial population in the diet. In contrast, however, no differences in LAB (Mikkelsen and Jensen, 1998) or even lower numbers of LAB (Mikkelsen and Jensen, 1997) were observed for piglets fed fermented diets compared with piglets fed non-fermented diets.

Feeding completely fermented diets reduced the number of Coliforms throughout the whole GIT (Mikkelsen and Jensen, 1997, 1998). In contrast, this reduction was not observed in the present study (Chapter 6) in which a liquid diet added with 45% fermented wheat (FW) was used. This seem to be due to the fact that the number of Coliforms in the diet with 45% FW was 1000-fold higher than in the non-fermented diet, probably caused by the high numbers of Coliforms in FW (data not shown). Moran et al. (in prep.) suggest a fermentation length of 48 hours in liquid wheat. In the current trial, we focused on 24 hour fermentation of liquid wheat because that is more suitable for practical applications than a 48 hour fermentation.

The number of Coliforms in the stomach of piglets fed the fermented diet was remarkably lower than the number of Coliforms present in that fermented diet (Chapter 5). This is in line with observations of Mikkelsen and Jensen (1998). Moreover, the number of Coliforms in the stomach of piglets fed the fermented diet was even lower than that of the piglets fed the non-fermented diet. These results indicate that the Coliforms were inactivated rapidly in the stomach, probably due to the lower gastric pH and higher gastric level of lactic acid present when fermented diets were fed. This suggests that fermentation has a negative effect on the presence of Coliforms in the GIT.

Morphology in the GIT

The surface of the small intestinal mucosa consists of finger-like villi projecting into the intestinal lumen. These villi accomplish an additional increase of the mucosal surface. The upper part of the villi is equipped for digestive and absorptive function. It is well-known that weaning of piglets result in villous atrophy directly after weaning (Makkink, 1993; Pluske et al., 1996a,b; Van Beers-Schreurs, 1996; Verdonk et al., 1999). The results of the present study, confirmed the generally accepted idea that villous height was reduced at day 4 after weaning compared to the day of weaning. Several authors demonstrated that villous length was higher in piglets that consumed more food (Makkink, 1993; Pluske et al., 1997) directly

after weaning. Moreover, a positive correlation between daily weight gain and villous length was reported by Pluske et al. (1996a), Van Leeuwen et al. (2001) and also the results in the present study indicates a correlation (Chapter 5). Several studies showed that reduced energy intake, independent of diet composition, was the major cause of villous atrophy after weaning (Table 2; Verdonk et al., 1999). The theory that villous height depends on the feed or energy intake is called "luminal nutrition theory".

Table 2. The relative effect of early post weaning energy intake on average villous height in the small intestine of pigs (adapted from Bruininx et al., 2000)

Author[1]	Energy source	Energy intake	Weaning	Slaughter	Villous height[2]
A	starter diet	5.7 MJ GE[3]/d	28 d	33 d	-30 %
A	ewes' milk	7.4 MJ GE/d	28 d	33 d	-2 %
B	cows' milk	2.3 MJ GE/d	29 d	34 d	-27 %
B	starter diet	5.1 MJ GE/d	29 d	34 d	-18 %
B	cows' milk	5.2 MJ GE/d	29 d	34 d	-4 %
B	cows' milk	8.9 MJ GE/d	29 d	34 d	+11 %
C	starter diet	0.53 MJ ME[4]/$BW^{0.75}$/d	28 d	32 d	-40 %
C	sows' milk	0.48 MJ ME/$BW^{0.75}$/d	28 d	32 d	-35 %
C	sows' milk	1.40 MJ ME/$BW^{0.75}$/d	28 d	32 d	-11 %

[1] A: Pluske et al. (1996a) ; B: Pluske et al. (1996b) ; C: Van Beers-Schreurs (1996)
[2] Villous height at weaning is expressed as 100%
[3] GE = Gross Energy
[4] ME = Metabolizable Energy

An important target of the present study was to investigate our hypothesis that a liquid diet added with fermented liquid wheat positively influence villous architecture, as a tool to improve growth performance directly after weaning. To prevent confounding of feed and / or energy intake with villous architecture, the amount of energy intake was calculated on average metabolic weight. The results clearly demonstrated that piglets fed the liquid diet with fermented wheat had higher villous height, higher villous to crypt ratio and better villous shape (Chapter 5) compared to the piglets fed the non-fermented diet. Therefore, feeding a diet added with fermented wheat seem to positively affect the villous architecture in the first part of the small intestine directly after weaning. Further, it can be concluded that besides the importance of energy or feed intake (Makkink, 1993; Pluske et al., 1996a,b; Van Beers-

Schreurs, 1996; Verdonk et al., 1999) also the type of nutrient seem to contribute to maintain villous architecture after weaning.

EFFECTS ON GROWTH PERFORMANCE AND ENERGY VALUE

Growth performance

Research on the impact of fermented diets has mainly been focused on completely fermented compound diets, whereas hardly any attention has been given to the addition of individually fermented carbohydrate-rich feedstuffs. Our study focused on the new concept of adding a fermented carbohydrate-rich feedstuff to a liquid diet and examine the effects on growth performance (Chapter 6).

Feeding completely fermented compound diets to weaned piglets affects positively daily feed intake and daily weight gain, whereas feed to gain ratio was negatively affected compared to non-fermented diets (Russell et al., 1996; Jensen and Mikkelsen, 1998; Pedersen, 2001). Pedersen (2001) studied the concept of mixing a proportion of fermented compound diet with non-fermented compound diet, the so-called "partly-fermented" compound diet. This concept resulted in a negative influence on feed to gain ratio (Pedersen, 2001), which is in line with the experiments done with completely fermented compound diets. One of the most reasonable explanations for the negative effect of completely or partly fermented compound diets on the feed to gain ratio is the breakdown of protein and amino acids into biogenic amines and ammonia. Pedersen (1999, 2001) reported higher levels of biogenic amines and higher loss of lysine in fermented compound diets compared with non-fermented compound diets. It was hypothesised by Rijnen and Scholten (1998) that using carbohydrate rich feedstuffs for fermentation is a more favourable fermentation concept than using complete compound diets. This hypothesis is strengthend by the results of the current study (Chapter 5+6). First, the addition of a fermented carbohydrate rich feedstuff (i.e., wheat) to a liquid diet, improved the performance, especially a better feed to gain ratio (Chapter 6). Second, fermented liquid wheat had a higher level of lysine compared with non-fermented wheat (Chapter 6). Therefore, it is suggested that the concept of adding fermented carbohydrate rich feedstuffs to liquid diets is more favourable for performance traits than the fermentation of complete compound diets.

Between the addition of 15%, 30, and 45% fermented wheat no differences in daily weight gain and feed to gain ratio were observed. This may implicate that the level of fermentation products in the 15% FW diet was high enough to obtain better performance traits. The piglets fed the 15% FW diet had an average daily consumption of 6.6, 1.2 and 1.0 g lactic acid, acetic acid and ethanol, respectively (Chapter 6). It is also possible that the potential benefit of higher lactic acid levels on feed intake could be not used because of the restricted feed intake. Several trials showed that the addition of lactic acid to pig diets increased the voluntary feed consumption and consequently daily gain (see review in Chapter 1). Therefore, it might be suggested that due to the restricted feeding level used in the current study no dose-response effect of the addition of fermented wheat on growth performance was observed.

Energy value

In the Dutch Net Energy Calculation for pigs (NEv) it is assumed that starch, sugar, lactic acid, acetic acid and ethanol are completely digestible for pigs (CVB, 2001). The energy values are 13.5, 12.2, 11.5, 9.6 and 21.3 kJ NE/g for starch, sugar, lactic acid, acetic acid and alcohol, respectively (CVB, 2001). Based on the data of the changes in carbohydrates and fermentation products, the changes in net energy values were calculated (Table 3).

Table 3. Calculation of change in energy (kJ NEv/kg), based on the changes (g/kg) in starch, sugar, lactic acid (LA), acetic acid (AA) and ethanol (ETH), as described in chapters 2, 3 and 6

	Starch	Sugar	LA	AA	ETH	energy[1]
Liquid wheat starch	-2.9	-4.6	1.2	2.2	-0.1	-62.5
Potato steam peel	-19.2	+2.0	12.9	1.4	0.3	-66.6
Cheese whey	n.p.[2]	-13.0	8.1	0.2	0.8	-46.5
Wheat	-25.5	-2.8	11.1	1.4	2.9	-175.5

[1] Based on 13.5, 12.2, 11.5, 9.6 and 21.3 kJ NE/g for starch, sugar, lactic acid, acetic acid and ethanol, respectively (CVB, 2001). Energy loss is expressed in kJ NE per kg product.

[2] n.p. = not present in cheese whey.

It is obvious that for all studied individual fermented feedstuffs, a loss of Net Energy (NEv) occurred during the fermentation process (Table 3). However, piglets fed a diet added with 45% fermented wheat, which had lower calculated energy level than non-fermented wheat (Table 3), showed higher daily gain and better feed to gain ratio than the piglets fed a diet with non-fermented wheat (Chapter 6). Based on these findings, it can be suggested that the

energy value of fermented wheat is higher than could be expected on the Net Energy calculation. This can be due to several reasons. The Dutch Net Energy formula is based on the digestibility coefficients of the nutrients and an assumed amount of energy needed for maintenance. First, it can be suggested that due to the soaking and fermentation of a feedstuff, the digestibility of some nutrients is improved compared to the non-fermented feedstuff. Indirectly this is proven by observations that the addition of organic acids to dry diets improved the total tract digestibility of protein and energy, and the absorption and retention of minerals, such as phosphorous (reviewed by Partanen and Mroz, 1999). In our study, after 48 hour fermentation of liquid wheat the level of phytic-acid phosphorous was 97% lower compared to the level of non-fermented wheat (data not shown). Until now, however, no experiments are published wherein the digestibility of non-fermented and fermented feedstuffs are compared. Second, the improved villous architecture in the first part of the small intestine of fermented diet-fed piglets (Chapter 5) probably result in less energy loss due to recovery and consequently piglets can use more energy for gain. Third, the fermentation products might alter the behaviour of animals, as suggested by Schrama et al. (1997). Pigs fed diets rich in non-starch polysaccharides, and consequently having high levels of fermentation products in the large intestine, use less energy for physical activity (Schrama et al., 1996, 1998). Fourth, the costs for digestion might be lower because of the pre-digestion (i.e., fermentation) of the feed before it is consumed by the piglet. As described before, it might be suggested that fermented diets needs less gastric HCL and less pancreatic secretion to optimise pH for digestion processes.

Based on the results obtained in the present study, it can be concluded that the reduction of energy loss due to the transformation of carbohydrates during fermentation is higher than the increase of energy yielded by the formation of the fermentation products lactic acid, acetic acid and ethanol. However, results on performance show that the "true" energy value might be higher than expected.

CONCLUSIONS

The main conclusions to be drawn from this thesis are:

- During storage of liquid diets for pigs the carbohydrates starch and sugar are transformed into the fermentation products lactic acid, acetic acid and ethanol. As a consequence there is a drop in pH-value and acid binding capacity during fermentation.
- The fermentation process of the liquid co-products is different from the fermentation process of the liquid compound diets. Liquid compound diets had a time-lag of 48 to 72 hours before they reached a pH below 4.5, whereas liquid co-products have a pH below 4.5 at the moment they are delivered to the farmers already.
- Liquid compound diets needs about 36 to 48 hours before they reach levels of lactic and acetic acid as present in liquid co-products at the start of storage. The ethanol levels are higher in liquid compound diets, indicating they had a different fermentation process.
- By fermentation the microbial population of a diet changes into higher levels of lactic acid bacteria and to a lesser extent yeasts. Fermentation seem to be a method to reduce Coliforms in the gastrointestinal tract.
- The addition of fermented wheat to a weaner diet influences the pH in the stomach and first part of small intestine in a more desirable way.
- The addition of 45% fermented wheat to a liquid weaner diet was favourable for villous height, villous to crypt ratio and villous shape in the first part of the small intestine of weanling piglets. Adding fermented feedstuffs to weaner diets seem to be an option for maintaining villous architecture directly after weaning.
- The addition of fermented wheat to a liquid weaner diet results in a clear improvement of the feed to gain ratio. Also a numerically higher average daily gain is observed.
- Between the addition of 15, 30 or 45% fermented wheat no differences in daily gain and feed to gain ratio is observed. This may be pre-dominantly the result of the restricted feeding level used in this experiment.
- Fermentation of liquid diets for pigs results in a loss of gross energy. The average loss is between 1 and 3%. However, the results on performance show that the "true" energy value might be higher than expected.
- The addition of fermented carbohydrate-rich feedstuffs to liquid diets is an interesting new feeding concept for weanling piglets.

SUGGESTIONS FOR FURTHER RESEARCH

The experiments described in this thesis were focused on "white-spots" in the research of fermentation of diets for pigs. Attention was given to the effect of fermentation on the diet composition, gastrointestinal physiology, microbiology and morphology, and growth performance traits. Comparative studies were scarce or even lacking in literature. The obtained results in the present study suggests that the addition of fermented carbohydrate rich feedstuffs to liquid diets may be a concept to maintain performance and gastrointestinal health after weaning. Because of the complexity of fermented diets, and the broad area of possible modes of actions, integrated research is advisable.

The present study showed that there is a difference in fermentation processes between the tested products, reflected by different levels and proportions of the most important fermentation products lactic acid, acetic acid and ethanol. This may be due to the fact that fermentation of liquid pig diets is mostly a "natural" fermentation without controlling the microbial populations. In several studies the addition of a starter culture, consisting of lactic acid bacteria, to pig diets showed only an effect at the start of the fermentation (Geary et al., 1998; Jensen and Mikkelsen, 1998). It fastened the start-up of the fermentation. However, after 24 hours no differences in the number of lactic acid bacteria, yeasts and Coliforms populations were observed. In our study (Chapter 4) the addition of a starter culture to liquid wheat was not affecting the microbial populations after 24 or 48 hours fermentation. Because the type and amount of fermentation products influence the energy value of the diet, it is interesting to control that process. Because the fermentation products are influenced by the type of microbial population, more attention to the control of the microbial population should be given. Development of a desirable fermentation and controlling of the process (e.g., addition of starter cultures; temperature) during fermentation of pig diets is needed.

The new concept of adding a fermented carbohydrate rich feedstuff to a liquid weaner diet showed that the growth performance was clearly improved. Because of several reasons, the piglets were fed at a restricted feeding level. It is suggested that due to the restricted feeding level, no dose-response effect of the proportion of fermented wheat in the liquid diet on growth performance was observed. Fermented diets contain high levels of lactic acid, from which in literature is known that lactic acid increases voluntary feed intake of pigs. Because of the importance of feed intake on daily weight gain, additional research should examine the effect of *ad libitum* feeding of diets added with a fermented feedstuff on growth performance traits. It might be also interesting to examine besides wheat also other carbohydrate rich feedstuffs. Possibly, feedstuffs with high levels of components which normally are 'digested'

by fermentation in the large intestine, like non-starch polysaccharides, might be upgraded trough pre-fermentation before feeding to pigs.

The present study clearly demonstrated that despite the loss of gross energy due to the fermentation, the performance traits of the piglets fed a fermented diet was improved. Because of the importance of an accurate prediction of the feeding value, it is necessary to obtain more information about the effect of fermentation on the feeding value. The Dutch Net Energy formula is based on the digestibility coefficients of nutrients and an assumed level of energy needed for maintenance. Therefore, research has to be focused on the effect of fermentation on 1) the digestibility and absorption of nutrients, and 2) the level of energy needed for maintenance.

The observed positive effect of feeding fermented diet to weanling piglets on the villous architecture offers possibilities to develop a new feeding strategy. To unravel the modes of action of fermented diets additional research is necessary. The most specific characteristics of fermented diets are 1) high levels of lactic acid, acetic acid and ethanol, and 2) specific microbial composition. The effects of these characteristics (separate and in combination) on gastrointestinal characteristics, growth performance and physical activity have to be studied.

PRACTICAL IMPLICATIONS

During fermentation of liquid feedstuffs and liquid compound diets a loss of dry matter occurs. However, partly this is due to the volatility of fermentation products such as lactic acid, acetic acid and ethanol when oven-drying (103 °C) or infra-red techniques are used. The present study shows that especially in fermented feedstuffs it is important to make a correction for the dry matter (DM) analysed by oven-drying. At the start of the storage the difference between uncorrected and corrected DM is roughly between 0.1 and 0.8%, whereas after six days storage the difference in DM increased to roughly between 0.9 and 1.4%.

Fermentation of complete compound diets (protein rich) increase the risk of protein breakdown and production of undesirable components such as ammonia and toxic amines. Several studies showed a reduction of the lysine content and an increase of toxic amines. Because protein breakdown products are 1) harmful for the gastrointestinal tract of pigs, and 2) negatively influencing the nutritional value of the diet, the fermentation of protein rich diets (i.e., compound diets) is an undesirable method of fermentation. Our research show that the fermentation of the carbohydrate rich feedstuff wheat results in a higher level of lysine.

Despite the reduction of starch and sugar during the fermentation process, the loss of gross energy is restricted. However, this energy loss seem to be compensated because of the higher performance of the pigs. This thesis demonstrate that the addition of a fermented carbohydrate rich feedstuff (i.e., wheat) improved performance, in particular the feed to gain ratio. In addition, piglets fed the diet with fermented wheat had favourable villous architecture directly after weaning. Based on these findings it can be concluded that the new concept of adding a fermented carbohydrate rich feedstuff to a liquid feed offers perspectives for improving the performance of weanling piglets. However, in our experiments the hygienic and temperature circumstances were controlled stringent. For practical application it is important to achieve a fermented diet which has a desirable microbial population to prevent undesirable fermentation processes and to control the fermentation in such a way that the loss of energy is minimal and the level and proportion of the different fermentation products is optimal.

REFERENCES

Beers-Schreurs, H.G.M. van, 1996. The changes in the function of the large intestine of weaned pigs. Ph.D. thesis, Universiteit Utrecht, Faculteit der Diergeneeskunde, The Netherlands, 149 p.

Bruininx, E.M.A.M., Peet-Schwering, C.M.C. van de, Schrama, J.W., 2000. Individual feed intake of group housed weanling pigs; relations with health? In: The Weaner Pig, Book of Abstracts, BSAS Occasional Meeting, University of Nottingham, Nottingham, England.

CVB, 2001. Centraal Veevoederbureau. Veevoedertabel. Lelystad, the Netherlands.

Geary, T.M., Brooks, P.H., Morgan, D.T., Campbell, A., Russell, P.J., 1996. Performance of weaner pigs fed ad libitum with liquid feed at different dry matter concentrations. J. Sci. Food Agric. 72, 17-24.

Jensen, B.B., 1999. Impact of feed composition and feed processing on the gastrointestinal ecosystem in pigs. In: Nutrition and gastrointestinal physiology – today and tomorrow -, p. 43.56.

Jensen, B.B., and L.L. Mikkelsen, 1998. Feeding liquid diets to pigs. In: Garnsworthy, P.C., Wiseman, J. (Eds.), Recent Advances in Animal Nutrition. Nottingham University Press, Loughborough, 107-126.

Leeuwen, P. van, Esteve-Garcia, E., Meijer, J.C., Zijderveld, F.G. van, Mouwen, J.M.V.M., Verstegen, M.W.A., 2001. Effects of Virginiamycin, as feed additive, on histological parameters of the small intestine mucosa and on performance in piglets. In prep.

Loo, D.J.P.H. van de, Scholten, R.H.J., 1997. Bijproducten via de drinknippel bij gespeende biggen en vleesvarkens. Report P1.186 (in Dutch, with English summary), Research Institute for Pig Husbandry, Rosmalen, The Netherlands.

Makkink, C.A., 1993. Of piglets, dietary proteins and pranceatic proteases. Ph.D. thesis, Department of Animal Nutrition, Agricultural University, Wageningen, The Netherlands, 168 p.

Mikkelsen, L.L., and B.B. Jensen, 1997. Effect of fermented liquid feed (FLF) on growth performance and microbial activity in the gastrointestinal tract of weaned piglets. In: Laplace, J.P., Fevrier, C., Barbeau, A. (Eds.), Digestive Physiology in Pigs. E.A.A.P. publication No. 88, 26-28 May. Saint Malo, France, 639-642.

Mikkelsen, L.L., and B.B. Jensen, 1998. Performance and microbial activity in the gastrointestinal tract of piglets fed fermented liquid feed at weaning. J. Anim. Feed Sci. 7, 211-215.

Partanen, K., Mroz, Z., 1999. Organic acids for performance enhancement in pig diets. Nutr. Res. Rev. 12, 1-30.

Pedersen, A. Ø., Fisker, B.N., Hansen, C.F., Kjeldsen, N.J., 1998. Liquid feed for growing-finishing pigs under Danish conditions. Paper no. 2.5, presented 49th Annual Meeting of European Association for Animal Production, 24-27 August, Warsaw, Poland

Pedersen, A. Ø., 1999. Styret fermentering af vådfoder. Kongres for Svineproducenter, 26-27 Oktober, Kopenhagen, Denmark.

Pedersen, A.Ø., 2001. Fermented liquid feed for weaners. Report no. 510, The National Committee for Pig Production, Danish Bacon and Meat Council, Denmark.

Pluske, J.R., Williams, I.H., Aherne, F.X., 1996a. Maintenance of villous height and crypt depth in piglets by providing continuous nutrition after weaning. Animal Science 62, 131-144.

Pluske, J.R., Williams, I.H., Aherne, F.X., 1996b. Villous height and crypt depth in piglets in response to increases in the intake of cows' milk after weaning. Animal Science 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.

Prescott, L.M., and J.P. Harley, D.A. Klein. 1996. Microbiology. WCB Publishers, 935.

Ravindran, V., Kornegay, E.T., 1993. Acidification of weaner pig diets: a review. J. Sci. Food Agric. 62, 313-322.

Rijnen, M.M.J.A., Scholten, R.H.J., 1998. Fermentatie van bijproducten en brijvoeders tijdens opslag. Report P1.211 (in Dutch, with English summary). Research Institute for Pig Husbandry, Rosmalen, The Netherlands.

Russell, P.J., Geary, T.M., Brooks, P.H., Campbell, A., 1996. Performance, water use and effluent output of weaner pigs fed ad libitum with either dry pellets or liquid feed and the role of microbial activity in the liquid feed. J. Sci. Food Agric. 72, 8.

Scholten, R., Verdoes, N., 1997. The dutch benefit from a recycling role. Pigs, 13-2, 16-17.

Scholten, R.H.J., Hoofs, A.I.J., Verdoes, N., 1997. By-products related to performance and environmental characteristics at growing/finishing pigs. Proefverslag P1.187. Praktijkonderzoek Varkenshouderij, Rosmalen, The Netherlands (in Dutch with English summary).

Scholten, R.H.J., Peet-Schwering, C.M.C. van der, Hartog, L.A. den, Vesseur, P.C., Verstegen, M.W.A., 1998. Effect of liquid by-products on performance and health of pigs. Paper no. 2.2, presented 49th Annual Meeting of European Association for Animal Production, 24-27 August, Warsaw, Poland.

Scholten, R.H.J., Peet-Schwering, C.M.C. van der, Verstegen, M.W.A., Hartog, L.A. den, Schrama, J.W., Vesseur, P.C., 1999. Fermented co-products and fermented compound diets for pigs: a review. Anim. Feed Sci. Technol. 82, 1-19.

Schrama, J.W., Verstegen, M.W.A., Verboeket, P.H.J., Schutte, J.B., Haaksma, J., 1996. Energy metabolism in relation to physical activity in growing pigs as affected by type of dietary carbohydrate. J. Anim. Sci. 74, 2220-2225.

Schrama, J.W., Heetkamp, M.J.W., Bakker, G.C.M., Vught, P. van, Verstegen, M.W.A., 1997. Invloed van fermentatie van koolhydraten en vulling van maagdarmkanaal op de activiteit in relatie tot de energiestofwisseling bij vleesvarkens. (in Dutch). Landbouwuniversiteit Wageningen, Vakgroepen Veehouderij en Veevoeding, The Netherlands. 39 pp.

Schrama, J.W., Bosch, M.W., Verstegen, M.W.A., Vorselaars, A.H.P.M., Haaksma, J., Heetkamp, M.J.W., 1998. The energetic value of nonstarch polysaccharides in relation to physical activity in group-housed, growing pigs. J. Anim. Sci. 76, 3016-3023.

Smits, B., 1998. Effect van bewaring en droge stofbepaling op de chemische samenstelling en op de mate van vervluchtiging van vluchtige bestanddelen in vochtrijke enkelvoudige diervoeders (in Dutch). Rapport ID-DLO no. 98.031. Instituut voor Dierhouderij en Diergezondheid. Lelystad.

Thaela, M.-J., Jensen, M.S., Pierzynowski, S.G., Jakob, S., Jensen, B.B., 1998. Effect of lactic acid supplementation on pancreatic secretion in pigs after weaning. J. Anim. Feed Sci. 7, 181-183.

Verdonk, J.M.A.J., Spreeuwenberg, M.A.M., Bakker, G.C.M., Verstegen, M.W.A., 1999. Nutrient intake levels affects histology and permeability of the small intestine of newly weaned pigs. In: Proc. 8th Symposium on digestive physiology of pigs. Uppsala, Sweden.

Visek, W.J., 1972. Effects of urea hydrolysis on cell life-span and metabolism. Fed. Proc. Fed. Am. Soc. Exp. Biol. 31, 1178-1193.

Visek, W.J., 1978. The mode of growth promotion by antibiotics. J. Anim. Sci. 46, 1447-1469.

Ward, G.L., Moran, C.A., Miller, B.G., Brooks, P.H., and Thorpe, J., 2001. Liquid feeding of newly weaned pigs: 1. The effects of feeding frequency and fermentation of liquid feed on growth performance and gastrointestinal morphology. In prep.

Winsen, R.L. van, Urlings, B.A.P., Lipman, L.J.A., Snijders, J.M.A., Keuzenkamp, D., Verheijden, J.H.M., Knapen, F. van, 2001a. Effect of fermented feed on the microbial population of the gastrointestinal tract of pigs. Appl. Environm. Microbiol., July 2001, 3071-3076.

Winsen, R.L. van, Lipman, L.J.A., Biesterveld S., Urlings B.A.P., Snijders, J.M.A., Knapen, F. van, 2001b. Mechanism of Salmonella reduction in fermented pig feed. Submitted to Journal of the Science of Food and Agriculture.

Summary

INTRODUCTION

In the last 30 years major changes occurred in pig nutrition. With the increasing size of the pig farms, manual feeding was replaced by automatic feeding systems. Most pigs were fed on dry diets, but during last the 15 years a clear tendency towards liquid feeding systems was observed. In the early 80's the use of liquid co-products from the human food industry was introduced into pig nutrition. In the Netherlands, nowadays about 20% of the slaughter pigs and about 10% of the sows is fed a liquid diet with one or more liquid co-products. On a yearly base 2.8 million tonnes of liquid co-products are re-used in Dutch pig nutrition. About ¾ of these liquid co-products can be classified as carbohydrate rich, which ferment during storage. Several data sources showed that pigs fed fermented liquid co-products had increased daily gain, better feed to gain ratio and lower mortality rate compared to pigs fed a non-fermented diet. In addition, research with fermented completely compound diets (compound feed mixed with water and soaked for several hours to days) showed favourable results. In general, a higher feed intake and higher daily gain was observed when piglets were fed fermented compound diets compared with non-fermented compound diets. However, feed to gain ratio was affected negatively by feeding fermented compound diets.

In conclusion, fermentation of liquid diets for pigs seems to improve growth performance and animal health. Fermentation of liquid diets for pigs might be a new feeding concept, especially because they might be an alternative for the prophylactic use of anti-microbial growth promoters in pig diets. However, at the time the present research started, hardly any information was available on the fermentation of liquid pig feedstuffs and the effects of fermentation on diet composition and energy value, and the effect of fermented diets on the gastrointestinal characteristics and growth performance traits of pigs. The major objective of this thesis was to elucidate these gaps in our knowledge.

CHARACTERISTICS AND POSSIBLE EFFECTS OF FERMENTED DIETS (Review)

To investigate the current knowledge about the characteristics of fermented feedstuffs and their modes of actions, a review was carried out (Chapter 1). When fermented liquid co-

products or liquid diets are fed to pigs, they have a pH-value between 3.5 and 4.4, whereas non-fermented diets have a pH-value between 5.5 and 6.1. Fermented feedstuffs have high levels of the fermentation products, like lactic acid (38.0 to 105.3 g/kg dry matter), acetic acid (3.6 to 35.3 g/kg dry matter) and ethanol (1.3 to 10.7 g/kg dry matter). The observed variability in chemical composition within the same product may be due to the different stages of fermentation of the sampled products. Knowledge on the changes in chemical composition during storage is scarce.

Literature showed, that the knowledge about the exact modes of action of fermented pig diets is scarce. However, the specific composition of fermented pig diets (i.e., higher levels of short chain fatty acids, lower pH, higher levels of lactic acid bacteria), makes it reasonable to suggest that the gastrointestinal tract (GIT) plays an important role. A limited number of studies showed that fermented diets are able to 1) reduce the gastric pH, 2) reduce the microbial activity in the GIT and 3) shift the microbial population in the GIT, of weanling piglets. Piglets fed a fermented diet had significantly lower numbers of Coliforms in the whole GIT. Some studies showed that short chain fatty acids (SCFA) positively influence intestinal epithelial proliferation. Moreover, the villous height is positively correlated with luminal levels of SCFA. Therefore, we hypothesized that fermented diets may positively influence the villous architecture in the small intestine of weanling piglets. Up till now, however, no published information is available about the effect of fermented diets on villous architecture.

FERMENTATION OF LIQUID CO-PRODUCTS AND LIQUID COMPOUND DIETS (Experiment 1)

Because knowledge on the changes in chemical composition and feeding value of liquid pig feed(stuffs) during storage is scarce, five different commonly used pig feedstuffs and diets were examined during a storage period of six days (Chapter 2 and 3). The studied products were three liquid co-products (liquid wheat starch, mashed potato steam peels, cheese whey) and two liquid compound diets (a grower diet, a finisher diet). The products were delivered directly from the production plant. The products were stored separately in 50-L lockable PVC tanks. Samples were taken at eight time-points during the storage period. This experiment was repeated three times.

During storage, the total amount of the carbohydrates starch and sugar decreased, whereas the total amount of the fermentation products lactic acid, acetic acid and ethanol

increased. At the end of the 6-day storage period, the total amount of the carbohydrates in both liquid compound diets decreased to 70% of the initial content, whereas for the liquid co-products the amount of carbohydrates decreased to between 73 and 94% of the initial content. During the 6-day storage, the total level of fermentation products (lactic acid, acetic acid, ethanol) increased to levels between 17.1 and 31.2 g/kg DM for the liquid co-products, whereas the liquid compound diets had levels between 36.2 and 40.4 g/kg DM. In all products a drop in pH-value and acid binding capacity was observed during the storage period. After the 6-day storage period, all products reached pH-values between 3.5 and 3.9. However, the liquid compound diets needed 48 to 72 hours to reach a pH-value below 4.5, whereas in the liquid co-products the pH-value was below 4.5 already at the moment of the start of the experiment. The acid binding capacity of the fermented liquid compound diets remained higher (between 156.6 and 161.2 meq 0.1 N HCL/kg) than that of the fermented liquid co-products (between 48.8 and 74.5 meq 0.1 N HCL/kg). In this experiment, there was no remarkable effect of the fermentation on the loss of gross energy. All products showed less than 3% energy loss after six days of storage.

FERMENTATION OF LIQUID WHEAT
(Experiment 2)

In the experiment described above, it was clear that liquid compound diets had a prolonged lag-phase (between 48 and 72 hours) before the pH-value reached a value below 4.5. In general, a pH higher than 4.5 gave favourable conditions for the multiplication of harmful microbes such as Coliforms. These are well-known as main producers of biogenic amines and ammonia. Danish trials showed clearly that the fermentation of complete compound diets resulted in a high loss of synthetic lysine (up to 30%) and a high production of biogenic amines. Therefore, in the next studies we focused on the fermentation of a carbohydrate rich feedstuffs: wheat (Chapter 4).

This experiment was carried out to examine the effect of a starter-culture and the proportion of pre-fermented wheat on the microbial and chemical composition of the liquid wheat. Liquid wheat (LW) was prepared by hammer-milling dry wheat and mixing with water (1:3 wt/wt) at 30° C. Five fermentation treatments were conducted in 45-L lockable PVC storage tanks housed in a room at 24 ± 1 °C, and stored for 48 hours. The Control treatment consisted of LW with no additions. Four further treatments investigated the benefits of inoculating the LW

or the addition of previously fermented LW (back-slopping). The 0% back-slop treatment (0% BS) was inoculated with 10 ml each of an overnight culture of *Lactobacillus plantarum* and *Pediococcus pentosaceus* (both from Alltech Inc., Kentucky, USA). In the three other treatments pre-fermented LW, prepared as above, was added to freshly prepared LW so that the pre-fermented wheat comprised 20, 33 or 42% of the final fermentation mixture (20% BS, 33% BS, 42% BS respectively). At times 0, 24 and 48 samples were taken to analyse the microbial and the chemical composition of the diets.

The effect of the starter culture was marginal, whereas back-slopping accelerated the acidification of liquid wheat and was essential to eliminate the Coliforms within the 48-hour steeping period. Liquid wheat reached in 24 hours a pH-value of 4.2 (without back-slopping) or 3.7 to 3.8 (with back-slopping). Between 20, 33 and 42% of back-slopping there were only slight differences in the microbial populations, and changes in the levels of carbohydrates and the levels of fermentation products. This study showed that hammer-milled wheat is a suitable raw material that allows quick fermentation.

IMPACT OF FERMENTED WHEAT ON GASTROINTESTINAL CHARACTERISTICS (Experiment 3)

Weaning of piglets is generally accompanied with villous atrophy. In our review (Chapter 1) we hypothesized that diets high in fermentation products might influence gastrointestinal characteristics, including villous architecture, of weanlings piglets. So far, however, hardly any attention has been given to the effect of fermented diets on gastrointestinal characteristics. In the experiment described above, it was shown that fermentation of liquid wheat is a good concept to achieve a fermented feedstuff within 24 hours. Therefore, liquid wheat was used in the following experiments to test the impact of a fermented feedstuff on the gastrointestinal characteristics (Chapter 5) and on growth performance (Chapter 6).

Gastrointestinal characteristics of 40, 28-d old weanling piglets were measured at the day of weaning, at day 4 and day 8 after weaning. Piglets were group-housed and fed twice a day. Feeding level was based on the average metabolic BW of piglets per group. Groups were fed a liquid diet with either 45% non-fermented wheat (FERM_0) or 45% fermented wheat (FERM_45). The other 55% of the diet was identical. To allow fermentation of the wheat, water of 30 °C was added to milled wheat, in a 2.2:1 ratio. Wheat was fermented by naturally present microbes for 24 h using a residue of 20% fermented wheat from a previous batch as

inoculum. The pH and contents of lactic acid, acetic acid, propionic acid and butyric acid were measured in the digesta of the stomach, three parts of the small intestine, ceacum and large intestine. In addition, changes in microbial populations in the digesta were studied during the period after weaning. Moreover, villous height, crypt depth and villous shape were studied in the small intestine.

The microbial composition of diet FERM_45 was different from diet FERM_0, reflected by higher numbers of lactic acid bacteria (LAB), yeast, Coliforms and E.coli. However, surprisingly, in the stomach of the piglets fed FERM_45 only the numbers of LAB and yeast were higher than for piglets fed FERM_0, at both d4 and d8. The number of Coliforms and E.coli were comparable between both treatments.

he piglets fed FERM_45 showed a lower gastric pH-value at d4 (3.6 versus 4.1), and a higher gastric lactic acid content at both d4 (86.22 versus 24.53 mmol/l digesta) and d8 (67.85 versus 23.18 mmol/l digesta) after weaning compared with piglets fed FERM_0. The piglets fed FERM_45 showed in the first part of the small intestine a higher villous height at d4 (321 versus 278 µm) and d8 (398 versus 295 µm), and a higher villous to crypt ratio compared with piglets fed FERM_0. Villous shape tended to be better for the piglets fed FERM_45. The present study shows clearly that the feeding of a liquid diet added with a fermented carbohydrate rich feedstuff might be a concept to minimize the undesirable changes in villous architecture post weaning.

IMPACT OF FERMENTED WHEAT ON GROWTH PERFORMANCE (Experiment 4)

To examine the effect of the addition of fermented liquid wheat to weanling diets on growth performance and animal health, a larger experiment was conducted (Chapter 6). Fifty-two pens of four 28-old weanling piglets were each fed one of the four experimental diets, which were similar in composition except for the content of fermented liquid wheat. The fermented wheat was prepared as in Experiment 3. Non-fermented wheat was exchanged for different doses of fermented liquid wheat (FW). Diets contained 0, 15, 30, or 45% FW. Piglets were given access to liquid feed twice per day. The restricted feeding level was calculated based on metabolic bodyweight of the piglets.

The 24-h fermented liquid wheat had lower pH-value (3.6 versus 5.9)) and a reduction of the starch and sugar contents, whereas the contents of lactic acid, acetic acid and ethanol increased compared with the non-fermented liquid wheat. The pH of the experimental diets was reduced by an increased addition of FW from 6.0 to 4.7. Piglets fed FW had numerical higher daily gain (+15 to +17 g/d) and improved feed to gain ratio (between 0.07 and 0.11) compared with the control diet. No dose-response effects of FW on growth performance traits were observed. The calculated daily gross energy intake was lowest for the piglets fed the diet with 45% FW. Despite this loss of energy, their daily gain was not negatively affected. This indicate that for these piglets the 'true' energy value of the fermented diet was higher than could be expected based on the nutrient composition. The present study shows that the addition of a fermented carbohydrate rich feed ingredient to a liquid diet for weanling piglets had positive effects on the daily gain and the feed to gain ratio.

CONCLUSIONS AND IMPLICATIONS

The major questions to be answered in the present study were:

1) whether fermentation is influencing chemical composition and feeding value of moisture rich pig diets;
2) whether a fermented diet is influencing gastrointestinal characteristics of weanling piglets;
3) whether feeding a fermented diet is influencing growth performance traits of weanling piglets.

Based on the results obtained from all experiments, it can be concluded that during fermentation a transformation of the carbohydrates starch and sugars into the fermentation products lactic acid, acetic acid and ethanol occurred. The pH-value and acid binding capacity of liquid co-products and liquid compound diets were reduced by fermentation. The fermentation process of liquid compound diets took 48 to 72 hours to reach a pH-value below 4.5, whereas liquid co-product had this pH-value already at the moment of delivery. The time-lag in pH-drop of liquid compound diets may explain the higher ethanol production in these diets. Liquid wheat reached within 24 hours a pH-value below 4.0.

An important question to be answered was the effect of a diet added with a carbohydrate rich feedstuff (i.e., wheat) on the gastrointestinal characteristics of weanling piglets. All piglets were fed based on average metabolic body weight, to prevent interaction between

feed intake and gastrointestinal parameters. In the present study, it was obvious that the diet high in fermentation products (45% fermented wheat) affected positively the gastric pH, gastric lactic acid content and the villous architecture in the first part of the small intestine.

The last question to be answered was the effect of a diet added with a carbohydrate rich feedstuff (i.e., wheat) on growth performance traits of restrictively fed piglets. It was obvious that the addition of fermented wheat to a liquid diet improved the growth performance, in particular the feed to gain ratio. Despite the lower average daily energy intake of the piglets fed the highest proportion of fermented wheat, they had a better performance than the control group. Between the tested range of 15 to 45% fermented wheat no effects on growth performance traits and animal health were observed. This might be pre-dominantly due to the restricted feeding level.

Based on the results of the present study, it can be concluded that the use of liquid diet with a fermented carbohydrate rich feedstuff has positive effects on the performance traits and the gastrointestinal health of weanling piglets compared with the use of non-fermented diets. It is recommended to examine and develop this new concept further, with also attention for the effects of *ad libitum* fed fermented diets and the modes of actions of fermented diets.

SAMENVATTING

Samenvatting

INTRODUCTIE

De varkensvoeding heeft de afgelopen drie decennia grote veranderingen ondergaan. Door de toename in bedrijfsgrootte, werd het handmatige voeren vervangen door automatische voersystemen. Het merendeel van de varkens krijgt droogvoer, hoewel de laatste 15 jaar een duidelijke tendens naar meer brijvoerinstallaties zichtbaar is. In het begin van de jaren '80 werd het gebruik van vochtrijke bijproducten uit de humane levensmiddelenindustrie in de varkensvoeding geïntroduceerd. Op het moment krijgt in Nederland circa 20% van de vleesvarkens en 10% van de zeugen een brijvoer met daarin één of meerdere vochtrijke bijproducten. Op jaarbasis wordt 2,8 miljoen ton vochtrijke bijproducten in de Nederlandse varkensvoeding hergebruikt. Circa ¾ van deze hoeveelheid kan worden aangemerkt als koolhydraatrijk bijproduct, die tijdens de opslag een fermentatieproces ondergaan. Diverse studies tonen aan dat varkens die een rantsoen met gefermenteerde vochtrijke bijproducten verstrekt kregen, een hogere groei en betere gezondheid hadden dan varkens die een brijvoer zonder deze vochtrijke bijproducten verstrekt kregen. Proeven met gefermenteerde volledige mengvoeders (mengvoer dat gemengd is met water en daarna enkele uren tot dagen geweekt) geven ook positieve resultaten te zien. In het algemeen hebben biggen die een gefermenteerd brijvoeder kregen een hogere voeropname en hogere groei dan biggen die een niet-gefermenteerd brijvoer kregen. Echter, de voederconversie wordt door het verstrekken van een gefermenteerd volledig mengvoer duidelijk verslechterd.

Concluderend, de fermentatie van brijvoer voor varkens heeft mogelijk een positief effect op de groeiprestaties en gezondheid. De fermentatie van vochtrijke voeders is wellicht een nieuw voerconcept, met name omdat deze voeders mogelijk een alternatief zijn voor het profylactisch gebruik van anti-microbiële groeibevorderaars in het rantsoen van varkens. Op het moment dat dit onderzoek van start ging, was er slechts in zeer beperkte mate kennis aanwezig over het fermenteren van varkensvoeders, en het effect van fermentatie op de chemische samenstelling en de voederwaarde van het voer, en het effect van een gefermenteerd voer op de kenmerken van het maagdarmkanaal en de groeiprestaties van varkens. Het belangrijkste doel van deze dissertatie was het ophelderen van de kennisleemte.

KENMERKEN EN MOGELIJKE EFFECTEN VAN GEFERMENTEERDE VOEDERS

(Review)

Om de huidige kennis over de specifieke kenmerken van gefermenteerde voeders en hun werkingsmechanismen te inventariseren, werd er een literatuurstudie uitgevoerd (Hoofdstuk 1). Op het moment dat gefermenteerde vochtrijke bijproducten of brijvoeders aan varkens werden gevoerd, hadden deze reeds een pH van 3,5 tot 4,4. Niet-gefermenteerde voeders hebben daarentegen een pH van 5,5 tot 6,1. Gefermenteerde voeders bevatten hoge gehalten van de fermentatieproducten melkzuur (38,0 tot 105,3 g/kg drogestof), azijnzuur (3,6 tot 35,3 g/kg drogestof) en ethanol (1,3 tot 10,7 g/kg drogestof). De waargenomen variatie in de chemische samenstelling binnen producten is mogelijk het gevolg van het tijdstip van fermentatie waarop de monsters genomen zijn. Kennis over de veranderingen van de chemische samenstelling tijdens de opslag van vochtrijke voeders is beperkt.

De literatuur toont aan dat de kennis over de exacte werkingsmechanismen van gefermenteerde varkensvoeders beperkt is. Echter, de specifieke samenstelling van gefermenteerde voeders (o.a., hogere gehalten kort-ketenige vetzuren, lagere pH en hogere aantallen melkzuurbacteriën), maakt het logisch te veronderstellen dat het maagdarmkanaal een belangrijke rol speelt. Een aantal proeven toonde aan dat gefermenteerde voeders in staat zijn om 1) de pH in de maag te verlagen, 2) de microbiële activiteit van ongewenste microben in het maagdarmkanaal te verlagen en 3) de microbiële samenstelling in het maagdarmkanaal van gespeende biggen te wijzigen. Biggen die een gefermenteerd brijvoer kregen, hadden significant lagere aantallen Coliformen in het maagdarmkanaal. Enkele studies geven aan dat kort-ketenige vetzuren een positief effect hebben op de proliferatie van het darmepitheel. Bovendien is de villus hoogte in de dunne darm positief gecorreleerd met het gehalte aan kort-ketenige vetzuren in de chymus van de dunne darm. Daarom luidde onze hypothese dat gefermenteerde voeders een positief effect op de villus compositie (villus lengte, villus:crypt ratio; vorm van de villi) hebben. Echter, tot op heden zijn er geen publicaties verschenen waarin het effect van gefermenteerde voeders op de villus compositie is beschreven.

FERMENTATIE VAN VOCHTRIJKE BIJPRODUCTEN EN BRIJVOEDERS (Experiment 1)

Omdat er weinig informatie over de veranderingen in de chemische samenstelling en de voederwaarde tijdens de opslag van vochtrijke voeders gepubliceerd is, zijn er vijf voedermiddelen gedurende een opslagperiode van zes dagen onderzocht (Hoofdstuk 2 en 3). Het betrof drie vochtrijke bijproducten (vloeibare tarwezetmeel, gemalen aardappelstoomschillen, kaaswei) en twee brijvoeders (startbrij, afmestbrij). De brijvoeders bestonden uit standaard mengvoer gemengd met water. Alle producten waren rechtstreeks afkomstig van de productielocatie. De producten werden apart opgeslagen in PVC vaten van 50 l. Gedurende de opslagperiode van zes dagen werd elk product acht keer bemonsterd. Het experiment werd drie keer herhaald.

Tijdens de opslag nam het gehalte aan koolhydraten (zetmeel, suiker) af, terwijl de gehalten van de fermentatieproducten melkzuur, azijnzuur en ethanol toenamen. Aan het einde van de 6-daagse opslag, was het totale koolhydraten gehalte in de gefermenteerde brijvoeders gedaald tot 70% van de beginwaarde, terwijl in de gefermenteerde bijproducten het gehalte daalde tot tussen de 73 en 94% van de beginwaarde. Tijdens de 6-daagse opslag, nam het gehalte aan fermentatieproducten in de vochtrijke bijproducten toe tot waarden tussen de 17,1 en 31,2 g/kg drogestof, terwijl dit in de brijvoeders toenam tot waarden tussen de 36,2 en 40,4 g/kg drogestof. In alle producten daalde de pH en het zuurbufferend vermogen. Na de 6-daagse opslag bereikten alle producten een pH tussen 3,5 en 3,9. Echter, de brijvoeders hadden 48 tot 72 uur nodig om een pH lager dan 4,5 te bereiken, terwijl deze waarde in de vochtrijke bijproducten al bij de start van de opslagperiode was bereikt. Het zuurbufferend vermogen van de brijvoeders (tussen 156,6 en 161,2 meq 0.1 N HCL/kg) bleef hoger dan die van de vochtrijke bijproducten (tussen 48,8 en 74,5 meq 0.1 N HCL/kg). In deze proef was er een gering effect van het fermentatieproces op het berekende bruto energieverlies. In alle producten was het energieverlies minder dan 3% na een 6-daagse opslag.

FERMENTATIE VAN TARWEBRIJ (Experiment 2)

Uit het hierboven beschreven experiment bleek duidelijk dat brijvoeders, bestaande uit mengvoer en water, een langere tijd (48 tot 72 uur) nodig hadden alvorens de pH een

waarde lager dan 4,5 bereikte. In z'n algemeenheid vormt een pH boven de 4,5 optimale omstandigheden voor de vermenigvuldiging van schadelijke bacteriën als Coliformen. Deze staan bekend als belangrijke producenten van biogene aminen en ammoniak. Deense studies toonden duidelijk aan dat de fermentatie van brijvoeders, bestaande uit mengvoer en water, resulteerde in een aanzienlijk verlies aan synthetische lysine (tot 30%) en een hoge productie van biogene aminen. Daarom richten we ons in de hierna volgende experimenten op het fermenteren van een koolhydraatrijke grondstof: tarwe (Hoofdstuk 4).

Dit experiment werd uitgevoerd om het effect van een startcultuur en het aandeel reeds gefermenteerde tarwebrij op de microbiële en chemische samenstelling van tarwebrij te onderzoeken. De tarwebrij werd verkregen door droge tarwe, gemalen op een hamermolen, te mengen met warm water van 30 °C (water-voer verhouding van 3:1). Vijf behandelingen werden uitgevoerd in afsluitbare PVC opslagvaatjes van 45 liter. Deze vaatjes stonden in een ruimte bij 24 ± 1 °C, en de tarwebrij werd gedurende 48 uur opgeslagen. De controlebehandeling bestond uit tarwebrij zonder toevoegingen. De overige vier behandelingen werden gebruikt om het effect van een startcultuur en het aandeel reeds gefermenteerde tarwe (zogenaamde "back-slopping") te onderzoeken. De 0% back-slop behandeling (0% BS) werd opgeënt met een startcultuur bestaande uit *Lactobacillus plantarum* en *Pediococcus pentosaceus* (beide afkomstig van Alltech Inc., Kentucky, United States). In de drie overige behandelingen werd reeds gefermenteerde tarwebrij aan de vers bereide tarwebrij toegevoegd, zodat het aandeel reeds gefermenteerde tarwebrij 20, 33 of 42% van de uiteindelijke tarwebrij bedroeg. Na 0, 24 en 48 uur werden er monsters genomen om de microbiële en chemische samenstelling te bepalen.

Het effect van de toevoeging van de startcultuur op het fermentatieproces was gering. Daarentegen was het openten met reeds gefermenteerde tarwe succesvol in het versnellen van de pH-daling en het elimineren van de Coliformen gedurende de opslagperiode van 48 uur. De tarwebrij bereikte binnen 24 uur een pH van 4,2 (zonder back-slopping) of een pH van 3,7 tot 3,8 (met back-slopping). Tussen de behandelingen met 20, 33 en 42% back-slopping was er weinig verschil in de microbiële populaties en de veranderingen in de gehalten aan koolhydraten en fermentatieproducten. Deze studie toont aan dat gemalen tarwe een geschikte grondstof is voor een snelle fermentatie.

IMPACT VAN GEFERMENTEERDE TARWE OP HET MAAGDARMKANAAL (Experiment 3)

Over het algemeen gaat het spenen van biggen gepaard met villus atrofie. In de literatuurstudie (Hoofdstuk 1) beschreven we onze hypothese dat voeders met een hoog gehalte aan fermentatieproducten mogelijk een positief effect hebben op de kenmerken van het maagdarmkanaal, inclusief de villus compositie, van gespeende biggen. Echter, tot nu toe is er nauwelijks informatie gepubliceerd over het effect van gefermenteerde voeders op de kenmerken van het maagdarmkanaal. In het hierboven beschreven experiment, bleek dat de fermentatie van tarwebrij een bruikbaar concept is om binnen 24 uur een gefermenteerd voedermiddel te hebben. Daarom hebben we in de hierna volgende experimenten gebruik gemaakt van gefermenteerde tarwebrij om daarmee de consequenties van deze gefermenteerde grondstof op de kenmerken van het maagdarmkanaal (Hoofdstuk 5) en op de groeiprestaties en gezondheid (Hoofdstuk 6) te onderzoeken.

Op de dag van spenen, dag 4 en dag 8 na spenen werd het maagdarmkanaal van in totaal 40 gespeende biggen (28 d leeftijd bij opleg) onderzocht. De biggen werden in groepen gehuisvest en twee keer per dag gevoerd. Het voerniveau was beperkt en gebaseerd op het gemiddelde metabool gewicht van een hok biggen. De dieren kregen een brijvoer met 45% niet-gefermenteerde tarwe (FERM_0) of een brijvoer met 45% gefermenteerde tarwe (FERM_45) verstrekt. De overige 55% van het voer was identiek voor beide groepen. Tarwebrij werd verkregen door gemalen tarwe te mengen met warm water van 30 °C in een water:voer verhouding van 2,2:1. Daarna werd de tarwebrij door de van nature in de tarwe aanwezige microflora gedurende 24 uur gefermenteerd. Tevens werd er voor gezorgd dat er altijd circa 20% reeds gefermenteerde tarwebrij (back-slopping) uit de vorige fermentatiebatch als startcultuur aanwezig was. De pH en de gehalten melkzuur, azijnzuur, propionzuur en boterzuur werden gemeten in de chymus van de maag, dunne darm (drie segmenten), blinde en dikke darm. Ook de microbiële populatie van de chymus werd bestudeerd. Er werden dunne darm biopten genomen om de villus lengte, crypt diepte en vorm van de villi vast te stellen.

De microbiële samenstelling van FERM_45 was verschillend ten opzichte van FERM_0. Dit kwam tot uiting in een hoger aantal melkzuurbacteriën, gisten, Coliformen en *E.coli* in FERM_45. Echter, het was opvallend dat in de maag van de biggen die FERM_45 kregen verstrekt, alleen nog de aantallen melkzuurbacteriën en gisten hoger waren. Het aantal Coliformen en E.coli was gelijkwaardig tussen beide behandelingen.

Het bleek dat biggen die FERM_45 verstrekt kregen een lagere pH op dag 4 na spenen (3,6 versus 4,1) en een hoger gehalte melkzuur op dag 4 (86,22 versus 24,53 mmol/l chymus) en dag 8 (67,85 versus 23,18 mmol/l chymus) in de maag hadden dan de biggen die FERM_0 kregen. De biggen die FERM_45 verstrekt kregen, hadden een hogere villus lengte op dag 4 (321 versus 278 µm) en dag 8 (398 versus 295 µm) na het spenen. Ook gaf het FERM_45 voer een hogere villus:crypt ratio in het eerste deel van de dunne darm. Bovendien werd in het eerste deel van de dunne darm een tendens tot een betere vorm van de villi geconstateerd indien de biggen FERM_45 kregen verstrekt. Dit experiment geeft duidelijk aan dat het voeren van een brijvoer met daarin een gefermenteerde koolhydraatrijke grondstof een geschikt concept kan zijn in het beperken van ongewenste veranderingen in de villus compositie direct na het spenen.

IMPACT VAN GEFERMENTEERDE TARWE OP GROEIPRESTATIES (Experiment 4)

Om het effect van de toevoeging van gefermenteerde tarwe aan brijvoeders voor gespeende biggen goed te bestuderen, werd een groter experiment uitgevoerd (Hoofdstuk 6). In totaal werden 52 hokken met vier gespeende biggen (28 d leeftijd) gebruikt om het effect van vier proefvoeders te bestuderen. De vier proefvoeders hadden een identieke samenstelling, uitgezonderd het aandeel gefermenteerde tarwebrij. De tarwebrij werd op een identieke wijze als in experiment 3 klaar gemaakt. Niet-gefermenteerde tarwe werd uitgewisseld tegen gefermenteerde tarwe (FW). De brijvoeders bevatten 0, 15, 30 of 45% FW. De biggen werden tweekeer per dag gevoerd. Het voerniveau was beperkt en gebaseerd op het gemiddelde metabool gewicht van een hok biggen.

De 24-uur gefermenteerde tarwebrij had een lagere pH (3,5 versus 5,9) en lagere gehalten aan zetmeel en suiker, terwijl de gehalten aan melkzuur, azijnzuur en ethanol toenamen in vergelijking tot de niet-gefermenteerde tarwebrij. De pH van de proefvoeders daalde door een toenemend aandeel FW van 6,0 naar 4,7. Biggen die een brijvoer met gefermenteerde tarwebrij verstrekt kregen, hadden een hogere dagelijkse groei (+15 tot +17 g/d) en duidelijk betere voederconversie (tussen 0,07 en 0,11) in vergelijking tot de controlegroep. Het aandeel gefermenteerde tarwe (15%, 30% of 45%) had geen effect op de groeiprestaties. De berekende dagelijkse bruto energie opname was het laagst bij de biggen die een rantsoen met 45% gefermenteerde tarwe kregen. Ondanks het verlies aan energie, werd de dagelijkse groei van de biggen niet negatief beïnvloedt. Dit duidt erop dat voor deze

biggen de "werkelijke" energiewaarde van gefermenteerd voer hoger was dan verondersteld mocht worden op basis van de nutriëntensamenstelling. Dit experiment toont aan dat de toevoeging van een gefermenteerde koolhydraatrijke grondstof aan een brijvoer voor gespeende biggen een positief effect op de dagelijkse groei en voederconversie heeft.

CONCLUSIES EN IMPLICATIES

De belangrijkste vraagstellingen van deze studie waren:

1) of fermentatie invloed heeft op de chemische samenstelling en de voederwaarde van vochtrijke varkensvoeders;
2) of een gefermenteerd voer invloed heeft op de kenmerken van het maagdarmkanaal van gespeende biggen;
3) of een gefermenteerd voer invloed heeft op de groeiprestaties van gespeende biggen.

Gebaseerd op de resultaten verkregen uit de vier experimenten, kan geconcludeerd worden dat er tijdens de fermentatie een belangrijke verschuiving van de koolhydraten zetmeel en suiker in de fermentatieproducten melkzuur, azijnzuur en ethanol optreedt. De pH en het zuurbindende vermogen van de bestudeerde vochtrijke bijproducten en brijvoeders namen af tijdens de fermentatie. Tevens bleek dat brijvoeders, bestaande uit mengvoer en water, ongeveer 48 tot 72 uur nodig hebben om de pH onder de 4,5 te laten zakken. Vochtrijke bijproducten hebben deze waarde al op het moment van afleveren bereikt. De vertraging van de pH-daling bij brijvoeders is mogelijk een verklaring voor de waargenomen hogere ethanolproductie in deze voeders. Tarwebrij bereikt binnen 24 uur een pH onder de 4,0.

Een belangrijke vraag van deze studie was of een brijvoer met daaraan toegevoegd een gefermenteerde koolhydraatrijke grondstof (tarwe) invloed heeft op de kenmerken van het maagdarmkanaal van gespeende biggen. Alle biggen werden beperkt gevoerd, op basis van het gemiddelde metabool lichaamsgewicht. Hiermee werd een interactie tussen de voeropname en de kenmerken van het maagdarmkanaal voorkomen. In de huidige studie, bleek duidelijk dat een brijvoeder met een hoog gehalte aan fermentatieproducten (45% gefermenteerde tarwe) een positief effect heeft op de pH en het melkzuurgehalte in de maag, en de villus compositie in het eerste deel van de dunne darm van gespeende biggen.

De laatste vraag die in deze studie beantwoord moest worden, had betrekking op het feit of een brijvoer met daaraan toegevoegd een gefermenteerde koolhydraatrijke grondstof (tarwe) invloed heeft op de groeiprestaties van beperkt gevoerde gespeende biggen. Het

was duidelijk dat de toevoeging van gefermenteerde tarwebrij aan het brijvoer een positief effect had op de groeiprestaties, in het bijzonder de voederconversie. Ondanks de lagere gemiddelde dagelijkse energie-opname van de gespeende biggen die het hoogste aandeel gefermenteerde tarwebrij kregen, realiseerden deze biggen een betere groei en voederconversie dan de controlegroep. De onderzochte range van 15% tot 45% gefermenteerde tarwebrij bleek geen effect op de groeiprestaties en gezondheid te hebben. Dit is mogelijk voornamelijk het gevolg van het beperkt voeren van de biggen.

Gebaseerd op deze resultaten kan worden gesteld dat het gebruik van een brijvoer met daaraan toegevoegd een gefermenteerde koolhydraatrijke grondstof een duidelijk positief effect heeft op de groei én de gezondheid van het maagdarmkanaal van pas gespeende biggen. Dit in tegenstelling tot brijvoer waaraan geen gefermenteerde grondstof is toegevoegd. Het verdient aanbeveling om dit nieuwe voederconcept verder te bestuderen en te ontwikkelen. Daarbij dient er ook aandacht te zijn voor de effecten van *ad lib* gevoerde gefermenteerde voeders en de exacte werkingsmechanismen van gefermenteerde voeders.

Publications of the author

Refereed scientific papers

Scholten R.H.J., Van der Peet-Schwering C.M.C., Verstegen M.W.A., Den Hartog L.A., Schrama J.W., Vesseur P.C., 1999. Fermented wet by-products and fermented wet compound diets for pigs: a review. Anim. Feed Sci. Technol. 82, 1-19.

Scholten R.H.J., Rijnen M.M.J.A., Schrama J.W., Boer, H.,. Den Hartog L.A., Vesseur P.C., Van der Peet-Schwering C.M.C, Verstegen M.W.A., 2001. Fermentation of liquid co-products and liquid compound diets: Part 1. Effects on chemical composition during a 6-day storage period. J. Anim. Physiol. Anim. Nutr. 85, 111-123.

Scholten R.H.J., Rijnen M.M.J.A., Schrama J.W., Boer, H., Van der Peet-Schwering C.M.C., Den Hartog L.A., Vesseur P.C., Verstegen M.W.A., 2001. Fermentation of liquid co-products and liquid compound diets: Part 2. Effects on pH, acid binding capacity, organic acids and ethanol during a six-day storage period. J. Anim. Physiol. Anim. Nutr. 85, 124-134.

Moran C.A., Scholten R.H.J., Brooks P.H., Verstegen M.W.A., 2001. Fermentation of liquid wheat: effects of the addition of a starter culture and different proportions of pre-fermented wheat. Submitted to Anim. Feed Sci. Technol.

Scholten R.H.J., Peet-Schwering C.M.C. van der, Schrama J.W., Hartog L.A. den, Verstegen M.W.A., 2001. Fermented wheat in liquid diets: effects on gastrointestinal characteristics in weanling piglets. Submitted to J. Anim. Sci.

Scholten R.H.J., Peet-Schwering C.M.C. van der, Schrama J.W., Hartog L.A. den, Verstegen M.W.A., 2001. Liquid diets for weanling piglets: effects of fermented wheat on growth performance and health. Submitted to Livest. Prod. Sci.

Conference papers

Scholten R.H.J., Verdoes N., Brakel C.E.P. van, Hartog L.A. den, 1996. Perspektiven von Nebenprodukten in der Schweinefütterung in den Niederlanden. Proceedings Workshop Unkonventionelle Futtermittel. 10/11 April 1996, Braunschweig, Deutschland, p. 66-70.

Scholten R.H.J., 1997. New Developments in Feeding Systems and Strategies in the Netherlands. Society of Feed Technologists, 5th November 1997, Kegworth, England.

Scholten R.H.J., Verdoes N., Hoofs A.I.J., 1998. By-products related to performance and environmental characteristics at fattening pigs. Proceedings Animal Production Systems and

the Environment, July 19-22, Des Moines, Iowa, United States of America, Volume 1: Oral Presentations, p. 109-114.

Scholten R.H.J., Peet-Schwering C.M.C. van der, Hartog L.A. den, Vesseur P.C., Verstegen M.W.A., 1998. Effect of liquid by-products on performance and health of pigs. Oral presentation at 49th E.A.A.P. European Association for Animal Production, 24-27 August 1998, Warsaw, Poland. Paper N2.2. (also published in Pig News and Information, 1999, 81N-88N).

Scholten R.H.J., The dose-response effect of fermented liquid wheat on growth performance and gastro-intestinal health of weaned piglets, WIAS-Science day, 29 February 2000, Wageningen.

Scholten R.H.J., Schrama J.W., Peet-Schwering C.M.C. van der, Hartog L.A. den, Vesseur P.C., Leeuwen P. van, Verstegen M.W.A., 2000. The dose-response effect of fermented liquid wheat on growth performance and gastro-intestinal health of weaned piglets. Congress Digestive Physiology in Pigs, 20-22 June 2000, Uppsalla, Sweden.

Scholten R.H.J., The use of liquid co-products and liquid diets in pig nutrition. Congress of FEDNA, 6-7 November 2000, Barcelona, Spain.

Research reports in field of liquid co-products and liquid diets

Brakel C.E.P. van, Scholten R.H.J., Backus G.B.C., 1996. Economische evaluatie van het voeren van natte bijproducten aan vleesvarkens. P1.147. Praktijkonderzoek Varkenshouderij, Rosmalen, Nederland.

Loo D.M.P.H. van de, Scholten R.H.J., 1997. Bijproducten via de drinknippel bij gespeende biggen en vleesvarkens. P1.186. Praktijkonderzoek Varkenshouderij, Rosmalen, Nederland.

Scholten R.H.J., Hoofs A.I.J., Verdoes N., 1997. Bijproducten in relatie tot technische resultaten en milieukenmerken bij vleesvarkens. P1.187. Praktijkonderzoek Varkenshouderij, Rosmalen, Nederland.

Scholten R.H.J., Hoofs A.I.J., Beurskens-Voermans M.P., 1997. Bijproductenrantsoen voor vleesvarkens: invloed van voerniveau en aminozurengehalte. P1.188. Praktijkonderzoek Varkenshouderij, Rosmalen, Nederland.

Hoofs A.I.J., Scholten R.H.J., 1998. Brijvoer via Vario-Mix of lange trog bij vleesvarkens. P1.206. Praktijkonderzoek Varkenshouderij, Rosmalen, Nederland.

Scholten R.H.J., Rijnen M.M.J.A., 1998. Het gebruik van vochtrijke bijproducten. Een literatuuroverzicht. P1.210. Praktijkonderzoek Varkenshouderij, Rosmalen, Nederland.

Rijnen M.M.J.A., Scholten R.H.J., 1998. Fermentatie van brijvoeders en bijproducten tijdens opslag. P1.211. Praktijkonderzoek Varkenshouderij, Rosmalen, Nederland.

Bruininx E.M.A.M., Scholten R.H.J., Binnendijk G.P., Roelofs P.F.M.M., Verdoes N., Haaksma J., 2000. Het toevoegen van perspulp aan droogvoer voor vleesvarkens. P1.245. Praktijkonderzoek Varkenshouderij, Rosmalen, Nederland.

Smolders M.A.H.H., Krimpen M.M. van, Scholten R.H.J., Loo D.J.P.H. van de, 2000. De invloed van melkzuur op de technische en financiële resultaten en gezondheid van gespeende biggen. P1.246. Praktijkonderzoek Varkenshouderij, Rosmalen, Nederland.

Contributions to symposia and meetings about liquid co-products and liquid diets

Scholten R.H.J., 1997. Actualiteiten Bijproducten. Schothorst Varkensdag 1997, Ede, Nederland, p. 43-46.

Scholten R.H.J., 1998. Fermentatie van vloeibare bijproducten en mengvoeders. Brijvoer cursus, FNM-VVM, 13 mei 1998, Arnhem, Nederland.

Scholten R.H.J., 1998. Ontwikkelingen op het gebied van bijproducten en voersystemen. Brijvoer cursus, FNM-VVM, 10 juni 1998, Arnhem, Nederland.

Scholten R.H.J., 1998. Het gebruik van vochtrijke bijproducten, losse granen en ruwvoeders in de varkenshouderij. PHLO-cursus 'Varkensvoeding: nieuwe ontwikkelingen en praktijk', Wageningen, 20 november 1998, Nederland.

Scholten R.H.J., 1998. Het voeren van bijproducten en granen aan varkens. Cursus Bijproducten MONAB, Lochem, 8 december 1998, Nederland.

Scholten R.H.J., 1999. Het gebruik van vochtrijke bijproducten en losse granen in de varkenshouderij. PHLO-cursus 'Varkensvoeding: nieuwe ontwikkelingen en praktijk', Wageningen, maart 1999, Nederland.

Scholten R.H.J., 1999. Het gebruik van vochtrijke bijproducten en losse granen in de varkenshouderij. PHLO-cursus 'Varkensvoeding: nieuwe ontwikkelingen en praktijk', Wageningen, november 1999, Nederland.

Scholten R.H.J., 1999. Het voeren van granen aan varkens. Studiedag ABC, Lochem, 28 juli 1999, Nederland.

Scholten R.H.J., 2000. Het gebruik van vochtrijke bijproducten en losse granen in de varkenshouderij. PHLO-cursus 'Varkensvoeding: nieuwe ontwikkelingen en praktijk', Wageningen, 7 december 2000, Nederland.

Scholten R.H.J., 2001. Vochtrijke diervoeders in de varkensvoeding. Brijvoedercursus Sondag Voeders, Erp, 23 en 25 januari 2001, Nederland.

Scholten R.H.J., 2001. Fermentatie van vochtrijke diervoeders. Studiedag Brijvoer, Denkavit Nederland B.V., Voorthuizen, 19 september 2001, Nederland.

Levensloop

Ronald Hendrik Johan Scholten werd op 2 juli 1969 te Enschede geboren. Hij groeide op in Glanerbrug. Na de lagere school (Gerhardus School) in Glanerbrug, ging hij naar het Jacobus College te Enschede. Aldaar behaalde hij het HAVO diploma. In 1986 begon hij met de studie Nederlandse Veehouderij – specialisatie Intensieve Veehouderij - aan de Hogere Agrarische School te Deventer. De praktijkcursus Hoger Kader Varkens was in het onderwijs programma van Deventer geïntegreerd. In 1990 begon hij aan het doorstroom programma van de Landbouwuniversiteit te Wageningen. In 1993 studeerde hij 'met lof' af in de Zoötechniek, met als hoofdvakken Reproductie & Gezondheidsleer, en Fysiologie van Mens en Dier.

Vanaf september 1993 tot en met augustus 1999 was hij werkzaam als onderzoeker "bijproducten en brijvoer" bij het Praktijkonderzoek Varkenshouderij te Rosmalen. Aldaar werden ook de proeven voor zijn promotieonderzoek uitgevoerd. Voor zijn onderzoek kreeg hij in 1998 de 'Young Scientist Award' van de European Association of Animal Production, sectie Nutrition. In september 1999 werd hij Hoofd Diervoeding & Kwaliteitszorg bij Beuker Vochtrijke Diervoeders B.V. te Doetinchem. Tot zijn taken behoort onder andere het geven van voedingstechnische ondersteuning van de productmanagers in Nederland, Duitsland, Engeland en Slowakije. Sinds augustus 2001 is hij woonachtig in Dánszentmiklós (Hongarije). Naast de bouw en het managen van een eigen varkensbedrijf (NOREL KFT), zal hij de werkzaamheden voor Beuker in Oost Europa gaan coördineren.

Het in dit proefschrift beschreven onderzoek is financieel mogelijk gemaakt door het Ministerie van Landbouw, Natuurbeheer en Visserij (MLNV) en de Productschappen Vee, Vlees en Eieren (PVE).

Verdere financiële bijdragen voor het drukken van het proefschrift zijn geleverd door:

ABCTA
Beuker Vochtrijke Diervoeders
Boerenbond Deurne
Cehave-Landbouwbelang
De Heus-Brokking-Koudijs
Denkavit Nederland
Purac Biochem
Sondag Voeders
Van Zelderen Fluid Handling
Weda

Printed by Universal Press, Veenendaal, The Netherlands.