Weaning of Pigs

Effects of Lectin Exposure and Weaning Strategies on Feeding Behaviour, Performance and Health

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Abstract

Post weaning diarrhoea is a common problem in pig production. The causes are multifactorial, but most often associated with infection of pathogenic strains of *Escherichia coli* in the small intestine. The aim of these studies was to investigate some of the physiological and managemental factors involved at weaning.

The lectin phytohaemagglutinin (PHA) is known for its growth promoting properties from studies on suckling rats and pigs. The hypothesis was that enteral exposure would induce precocious maturation, leading to a gastrointestinal (GI) tract better prepared for weaning. PHA-treated pigs grew slower during the treatment week before weaning, and in one of the two studies presented here; the PHA-treated pigs had a higher body weight (BW) gain than the control pigs in the week immediately following weaning. PHA treatment resulted in lower diarrhoea scores, a more beneficial feeding pattern and a tendency to greater feed intake. Effects on physiological parameters were shown, with a lower uptake of marker molecules, and a more adult disaccharidase pattern in PHA-treated pigs. No differences were found regarding immunological status, weight and length of the intestinal organs, the pancreatic enzyme activities or feed conversion ratio.

It was investigated how pigs of different relative size (small, medium and large) in a group of 10 pigs were affected by receiving feed from a feed dispenser with 2 feeding places per pen, or from a trough where all pigs could eat at the same time. Also, comparisons were made between keeping pigs as unmixed litters staying in their farrowing pen after weaning, or mixing and moving them to a new pen on the day of weaning. Trough feeding resulted in lower diarrhoea scores for the small and large pigs, and higher scores for the medium pigs. All size categories had longer feeding bouts when fed at a trough, and longer total feeding times on day 1 (small pigs) and 5 (large pigs) after weaning. The feed conversion ratio was lower during the second week after weaning for the pigs fed from feed dispensers. The mixing and moving procedure resulted in lower BW gain compared to that of pigs weaned as whole litters, which was most pronounced in small pigs. Mixed and moved pigs also had lower feed intakes and higher diarrhoea scores (small and large pigs).

Keywords: pig, weaning, body weight gain, feeding behaviour, post weaning diarrhoea, maturity, lectin, feed dispenser, trough, mixing

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To my family

*Whether you believe you can do a thing or not, you’re right.*

Henry Ford
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List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:


III Thomsson, A., Rantzer, D., Botermans, J. and Svendsen, J. The effect of feeding system at weaning on performance, health and feeding behaviour of pigs of different sizes (accepted for publication after revisions in *Acta Agriculturae Scandinavica, Section A, Animal Science*).

IV Thomsson, A., Botermans, J., Rantzer, D and Svendsen, J. The effects of mixing/moving and interaction with feeding system on performance, health and feeding behaviour of pigs having different sizes at weaning (submitted).

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### Abbreviations

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<tr>
<td>BSA</td>
<td>Bovine serum albumin</td>
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<td>BW</td>
<td>Body weight</td>
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<td>GI</td>
<td>Gastrointestinal</td>
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<td>FCR</td>
<td>Feed conversion ratio</td>
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<td>IgG</td>
<td>Immunoglobulin G</td>
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<td>NaF</td>
<td>Sodium fluorescein</td>
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<td>PHA</td>
<td>Phytohaemagglutinin</td>
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<td>PWD</td>
<td>Post weaning diarrhoea</td>
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Background

Weaning is the process by which the progeny becomes independent of their mother in terms of nutrition and protection. In nature, this process differs between the mammalian species; in pigs it is a gradual course of events over an extended period of time, where the piglets increase their search for, and consumption of feed from their environment, while they are still suckling. Little by little, the suckling frequency decreases, to finally cease at an average age of 17 weeks (Jensen and Recén, 1989).

Economic interests have a large impact on modern commercial pig production, and by decreasing the weaning age, the number of weaned pigs produced annually per sow have increased dramatically. Swedish law prohibits weaning before 28 days of age, and in the EU the lowest weaning age permitted is 21 days. In some countries, e.g., the USA, weaning ages as low as 10–14 days are common. Early weaning may have negative consequences, manifested as poor growth and PWD, because the pig’s GI tract is not fully developed at this young age.

Traditionally, PWD has been kept under control via supplementing the feed given after weaning with growth promoters/antibiotics, but their usage was banned in Sweden in 1986 and in the rest of the EU in 2006. After the ban, proper management has become more important in order to maintain good pig health, and the use of antibiotics for medication has slowly decreased. Still, PWD is a problem in many herds, and where disease can be expected, feed medication (with ZnO or antibiotics) can be prescribed by a veterinarian (Odensvik et al., 1999).

Research into the causes of PWD today concerns alternative in-feed additives, such as herbal preparations (Kommera et al., 2006), pre- or prebiotics or organic acids and their salts, quality and concentration of protein and carbohydrates in the feed (see review by Halas et al., 2007), and measures to improve management routines (Evans, 2001).
Introduction

Physiological Aspects of Weaning

Digestion

In the small intestine, nutrients from the feed are degraded and absorbed by the epithelial cells. The small intestinal surface is highly enlarged through folding and the formation of finger-like villi and crypts. The presence of microvilli, or brush border, on the luminal side of the epithelial enterocytes further increases the absorptive area. The enterocytes and other epithelial cell types originate from stem cells in the crypts, and proliferate and differentiate during their journey upwards the villi, to finally be sloughed off from the tip. This cell turnover takes 2-4 days, and continues throughout the life of the animal.

Following weaning, classical observations are a decrease in the villi heights and increase in the crypt depths (Cera et al., 1988; Hampson, 1986; Gay et al., 1976). This can be explained by an increased rate of cell loss associated with an increased crypt-cell proliferation, which leads to an increase in the crypt depth. The increase of crypt cell production is caused by environmental changes following weaning, such as the presence of a new diet and different luminal microflora (Pluske et al., 1997). Another explanation for the alterations in villi/crypt structure is the presence of intestinal inflammation due to inadequate feed intake immediately following weaning (McCracken et al., 1999).

Enzymes for feed digestion are produced in the saliva, stomach, pancreas, and small intestine. Secretions with pH regulating, buffering and fat solubilising properties also facilitate digestion. Development of the enzymatic profile is partly age-regulated, but also involves adaptation to the
composition of nutrients in the feed. This becomes apparent at weaning, when the nutrient source abruptly changes from milk to a solid, cereal-based diet. The disaccharidases, lactase, maltase and sucrase, are present in the brush-border of the small intestine. During the suckling period, lactase predominates, breaking down the lactose from milk; whereas maltase and sucrase activities not only increase with age (Aumaitre and Corring, 1978), but also in response to the substrate present, i.e., the carbohydrate composition of the feed (see review by Bach Knudsen and Jørgensen, 2001).

Immunology

Sow colostrum provides the piglets with maternal antibodies, which are absorbed as intact molecules into the blood serum before gut closure, which occurs approx. 12–24 h after suckling begins (Weström et al., 1985). These IgG antibodies are crucial for the pigs, since no maternal antibodies are transferred from the sow via the placenta to the fetus (Sterzl et al., 1966). The maternal IgG is protective against the many environmental pathogens which the sow has encountered; however, pathogens of risk for the piglets are often located on the intestinal mucosal surfaces, where there is little or no effect of IgG protection (Gaskins, 1997). Sow milk also contains IgA molecules, which provide local intestinal immune protection, adapted to the present environment (Klobasa et al., 1981). Gradually, the piglets build up their own repertoire of IgG antibodies, but at the time of early weaning, the level of circulating IgG is low (Saito et al., 1986; Klobasa et al., 1981), and at the same time IgA from milk is unavailable. Taken together, the weanling pig can be regarded as being poorly protected against the new challenges.

Feed Intake Characteristics

Pigs have the capability to grow 180–240 g per day between birth and weaning (Pluske et al., 1995). Weaning implies a drastic change in nutritional source, from that of milk to solid feed, and to ensure proper development of the GI tract and maintain gut integrity, it is of importance that the pig ingests feed after weaning in sufficient amounts (Pluske et al., 1996). This is also necessary to avoid a growth check, which can affect the total time from weaning to market, and thereby production economy. The GI tract responds to the new feed with the maturational processes described above, but there is a delay before the digestive enzymes reach efficient levels (Rantzer et al., 1997; Makkink et al., 1994; Cera et al., 1990). This makes it difficult to maintain equivalent levels of nutrition and energy for growth and development during the first days following weaning. Feed intake varies
significantly between individuals and litters; both in total level of intake, and the time before the pigs start eating after weaning (Bruininx et al., 2001).

In addition, the pigs are not accustomed to regulate their own feed intake just after weaning, since they were just recently dependent on the sow’s willingness to nurse. It may be assumed that just after weaning, the pigs’ ability to feel hunger and satiety, and thereafter regulate their feed intake, is not fully developed. This, in some cases, results in overeating, which can be seen in post-mortem examinations of pigs that died from PWD, having a stomach filled with undigested feed (Svendsen et al., 1974).

**Post Weaning Diarrhoea**

Gastrointestinal disturbances caused by microorganisms are common problems in pig production and PWD is the most frequently observed disease during the weeks immediately following weaning. It is strongly associated with pathogenic strains of *Escherichia coli*, which adhere to the small intestinal surface and produce enterotoxins, leading to severe secretory diarrhoea. Infected piglets react with growth depression, and risk dying from dehydration if they are not treated with antibiotics. Naturally, the sick piglets also suffer, leading to a welfare problem worth addressing, not just a problem of economic significance for the pig producer.

Although *E. coli*-associated, PWD must be regarded as being a multifactorial disease (Melin et al., 2000), since it appears under complex circumstances. At the time of weaning, the pig is in a developmental phase with regards to gut function and immunity, and is also under stress from being removed from the sow. The abrupt feed change from milk to solid feed, and immature digestive capacity before an efficient enzyme profile can be achieved, results in inadequately digested feed remaining in the GI tract, which is then available as a substrate for the growth of pathogenic *E. coli* (Kenworthy and Crabb, 1963). The ingredients (Pluske et al., 2001; Prohászka and Baron, 1980; Bertschinger et al., 1978/1979; among others) and structure (Brunsgaard, 1998) of the diet have been shown to affect the severity of the disease. Environmental factors are also of importance for the health status of the animals, e. g., pen hygiene (Rantzer et al., 1999; Madec et al., 1998) and environmental thermal conditions (Le Dividich and Herpin, 1994).
Induction of Gut Maturation by Phytohaemagglutinin Exposure

PHA is a lectin derived from red kidney beans. Most lectins are glycoproteins that can bind to carbohydrate groups on, for example, a cell surface. When ingested, PHA resists proteolysis, and passes undigested through the stomach. In the small intestine, PHA binds to the glycosylated surface of the villi epithelial cells (Baintner et al., 2000). After binding to the gut epithelium, PHA has powerful growth promoting properties, shown primarily in studies on rats (Linderoth et al., 2005a). PHA also has been shown to be able to induce maturational changes in the GI tract on suckling pigs and rats, resulting in the pancreas and small intestine having more adult-like properties, similar to those normally observed at weaning (Linderoth et al., 2006; Linderoth et al., 2005a,b; Rådberg et al., 2001). This has made PHA very interesting for use in experiments on pigs. Inducing a weaning-like development of the GI tract prior to the actual weaning would imply fewer simultaneous changes and perhaps reduce the impact of stress on the pigs on the day of weaning.

Management Routines

Feed Presentation

Feed dispensers with limited number of feeding places are commonly used in pig production because they are designed to minimize feed wastage, and because they allow for a more flexible pen design. Troughs, on the other hand, encourage social facilitation, which has proved to be of importance to pigs (Hsia and Wood-Gush, 1983) and allows for continuation of the group-feeding behaviour similar to the suckling situation. Extensive studies have been performed to investigate feed intake characteristics and growth performance, using different ways of feed presentation (O’Connell et al., 2002; Horváth et al., 2000; Hyun et al., 1998, Pluske and Williams, 1996). In most cases, pen mean values have been compared, whereas in the studies underlying this thesis, pigs of different relative sizes in the groups were studied separately. No previous studies had investigated the effect of different types of feed presentation on the occurrence of PWD.

Mixing and Moving Routines

Mixing piglets at weaning to form new groups and moving them to new pens is a common procedure at weaning. The mixing procedure alone causes stress due to the disruption in the rank order and the encounter with unfamiliar animals (Merlot et al., 2004), leading to an increase in aggressive
behaviour (Ekkel et al., 1995). Searching the literature showed that no previous studies have shown any effect of mixing on feeding behaviour (Dybkjær et al., 2006; Merlot et al., 2004). Pluske and Williams (1996) found that piglets mixed at weaning grew faster and consumed more feed during the first 14 days after weaning, in comparison to animals in unmixed litters. In growing pigs, however, studies have shown impaired growth as a result of mixing (Hyun et al., 1998; Lund et al., 1998; Sherritt et al., 1974). The present study of mixing and moving at weaning was performed not only to determine if the mixing/moving procedure affected pigs of different relative size in the group differently in terms of BW gain, but also to investigate the possible effect on the occurrence of PWD, which has not been reported previously.

Aims of the Thesis

The aims of the present thesis were to investigate:

- If enteral exposure to PHA one week before weaning would improve performance and health, in terms of BW gain and PWD occurrence (I, II),
- The effect of enteral exposure to PHA before weaning on feed intake characteristics post weaning (II),
- To what extent pigs of different body size categories in a group, mixed at weaning, react to being fed from a feeder or from a trough, with respect to BW gain, PWD occurrence and feed intake characteristics (III),
- To what extent pigs of different body size categories react to being mixed and moved at weaning, compared with staying with their littermates in the farrowing pen, with respect to BW gain, PWD occurrence and feed intake characteristics (IV), and
- If any interaction exists between the mixing/moving procedure and feeding system (feed dispenser/trough; IV).

The complexity of the weaning problem and the approaches of the different studies comprising this thesis are illustrated in Figure 1.
Figure 1. An overview of different factors that affect performance and health at weaning, and how the studies reported in the different papers in this thesis relate to the weaning problems.
Material and Methods

The 4 studies (Table 1) were conducted in an environment similar to a commercial Swedish pig farm, with respect to housing, animal material and infection pressure. The time period studied started 7 days before weaning (I, II). Observations were made most frequently during the 2 weeks immediately following weaning, and final observations were made on day 28 after weaning. For details of the methods, see the respective paper.

Animals, Housing and Management

All studies were performed at Odarslöv Research Farm, under the management of the Dept. of Rural Buildings and Animal Husbandry, Swedish University of Agricultural Sciences, Alnarp. The farm had a closed herd of 50 sows, and piglets were kept on the farm until slaughter (farrow to finish production). The sows farrowed in batches of 16-17, every 7-8\textsuperscript{th} week. Weaning took place at 31-34 days of age. Cross-bred ((Swedish Landrace x Yorkshire) x Hampshire) pigs (castrates and gilts) were used for the studies.

The pigs had \textit{ad libitum} access to the standard starter diet Pigfor Växett (Lantmännen, Stockholm, Sweden; content per kg: 13.0 megajoules metabolizable energy; 161 g crude protein; 63 g crude fat; 44 g crude fibre; 12.0 g lysine; 7.8 g methionine + cysteine; 7.0 g threonine) after weaning. The same feed was used as creep feed, given on the floor before weaning (III, IV). No antibiotics or other additives were given in the feed. Pigs with severe PWD were treated by antibiotic injections (Borgal vet., Intervet International, Schwabenheim, Germany) for 3 days.

The housing facilities where the studies were carried out was a stable with farrowing pens (3.35 x 2.10 m; slatted floor on 37\% of the total area; I, IV) which could also be used to house the litter after weaning up to approx. 25 kg live weight. There was a unit with specialized pens for weaning pigs.
(3.35 x 1.20 m; slatted floor on 37% of the total area; III-IV) in the same stable. In paper II, the pigs were housed in pens of 1 x 1 m with slatted floor on 75% of the area. They were fed once daily, in the morning; with amounts sufficient to last until the next morning. Feed dispensers (2 feeding places per pen) were used in paper I, feed dispensers and troughs (10 feeding places per pen) were used in paper III-IV. In paper II, feed dispensers with one feeding place per pen were used. Chopped straw was used as bedding material in the farrowing pens and weaning pens, and cutter shavings in the small pens. All pens had a heating lamp situated over the lying area and a water nipple situated over the slatted floor.

**Experimental Designs**

In papers I and II, a split litter design was used. Half of the piglets in each litter were treated with either PHA or the control protein α-lactalbumin. In paper I, piglets stayed in the pens where they were born, for the entire experimental period. In paper II, piglets were moved at weaning, into smaller pens of approx. 1 m², and kept in pairs with littermates belonging to the same treatment group. They remained in the small pens for 14 days, and were then returned to their farrowing pen. In paper III, the pigs in each group were mixed from at least 4 different litters into new groups of 10 pigs each, housed in specialized pens for weaners, and fed from either feed dispensers or troughs. Paper IV was set up as a 2 x 2 factorial design, where the first factor was being mixed and moved (into specialized weaning pens; same mixing procedure as in paper III) or not mixed or moved (i.e. staying with littermates in the farrowing pen), and the second factor was being fed from a feed dispenser or from a trough. On the day of weaning, the 3 lightest pigs in the group/litter were categorized as being small, the 3 heaviest were categorized as being large, and consequently, the 4 remaining were categorized as medium (III, IV).

**Lectin Preparation and Administration**

The crude lectin (PHA) solution used for the experiments in papers I and II was prepared from finely grounded red kidney beans (*Phaseolus vulgaris*), according to the method described by Pusztai and Watt (1974). Using a haemagglutination assay, the lectin content was established at approximately 25%. Besides lectin, the crude preparation mainly contained trypsin and amylase inhibitors, and tannins. Using a soft polyvinyl chloride stomach tube, the pigs were fed 400 mg crude PHA/kg of BW, dissolved in 0.9%
NaCl, on days -7, -6 and -5 in relation to weaning. Given the lectin content of 25%, the dose of PHA was estimated to be about 100 mg/kg BW.

Registrations

Body Weight Gain and Diarrhoea Scores
The piglets were weighed on several occasions during the experimental periods, starting one week before weaning (I, II), and ending 4 weeks after weaning (I-IV).

Diarrhoea scores were registered on most days during the two weeks after weaning, using the following scale: 0 = no signs of diarrhoea, 1 = a little loose faecal consistency, 2 = very loose faecal consistency, and 3 = watery, spurting faeces (Rantzer et al., 1996).

Feeding Behaviour
In all studies (but not all batches), pigs were videotaped using a time-lapse recorder and a surveillance camera, and the tapes from days 1 and 5 were used to register feeding behaviour. When analysing the tapes, a pig was considered to be eating when its nose was pointed downwards in the feed dispenser/trough. Eating had to last for at least 5 s to be registered, and interruptions during eating of less than 5 s were not registered. In order to evaluate differences between treatments, total time spent feeding, feeding time split into 4-h-intervals, and number and length of bouts were compared. One bout was defined as the sum of the feeding time where not more than 5 min had passed with the pig not eating.

Feed Intake
Feed intake was recorded by weighing the feed given each morning, and weighing the feed not consumed from the day before. No electronic feeder equipment was used, and thus no individual measures of actual feed intake could be performed. In papers II-IV, pigs in each treatment group were housed in different pens, so feed intake was recorded on the pen level. FCR was calculated for the periods days 0-7, 7-14 and 0-14, in relation to weaning (also on pen level) as kg of consumed feed/kg gain in BW.
<table>
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<th>Treatment</th>
<th>No. of pigs</th>
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<td>I</td>
<td>To induce precocious functional maturation of the GI tract by exposure to PHA, and to study its effect on important parameters in commercial pig production</td>
<td>PHA/α-lactalbumin</td>
<td>48</td>
<td>BW gain, Diarrhoea scores, Proportion of haemolytic E. coli, Feeding behaviour, Uptake of marker molecules, Organ lengths/weights, Enzymes in pancreas and small intestine, Total protein and IgG in plasma</td>
</tr>
<tr>
<td>II</td>
<td>To investigate the effect of exposure to PHA on feed intake characteristics.</td>
<td>PHA/α-lactalbumin</td>
<td>62</td>
<td>BW gain, Diarrhoea scores, Proportion of haemolytic E. coli, Total protein and IgG in plasma, Feeding behaviour, Feed intake, FCR</td>
</tr>
<tr>
<td>III</td>
<td>To evaluate the effects on performance and health of pigs of different relative size, when fed from different feeding systems.</td>
<td>Feed dispenser/trough</td>
<td>280</td>
<td>BW gain, Diarrhoea scores, Feeding behaviour, Feed intake, FCR</td>
</tr>
<tr>
<td>IV</td>
<td>To evaluate the effects on performance and health of pigs of different relative size, when fed from different feeding systems, and when being mixed and moved or not.</td>
<td>Feed dispenser/trough</td>
<td>157</td>
<td>BW gain, Diarrhoea scores, Feeding behaviour, Feed intake, FCR</td>
</tr>
</tbody>
</table>
Samples and Analyses

Blood Samples
In papers I and II, repeated blood samples were taken from the cranial vena cava using disposable syringes containing 1.5 mg of EDTA and 1000 kIU of Trasylol (Bayer, Leverkusen, Germany), on days -7, -5, 0, 2, 7, 14 and 28. The plasma samples were analysed for total protein according to Lowry et al. (1951), and for IgG with radial immunodiffusion (Fahey and McKelvey, 1965).

In paper I, a marker molecule solution containing 2 different marker molecules with different properties (BSA; Sigma, St. Louis, USA; with high molecular mass, and NaF; Merck, Darmstadt, Germany; with low molecular mass) was gavage fed on the day before weaning. To analyse the uptake from the small intestine, blood samples were obtained at 0 h and at 0.5, 1, 2, 4, 8 and 24 h after administration. Plasma levels of the marker molecules NaF and BSA were analyzed according to Nejdfors et al. (2001).

Bacteriology
Faecal samples, for bacteriology analyses, were taken for papers I and II on days -2, 0, 5, 7 and 14, by inserting a cotton swab into the rectum. The samples were dissolved in 0.85% NaCl solution, and analysed for the proportion of haemolytic E. coli with respect to the total aerobic bacteria, according to Rantzer et al. (1995).

Organ Sampling and Enzyme Analyses
In paper I, 6 PHA-treated and 6 control pigs were anaesthetised using an i.v. injection of a barbiturate solution. From the anaesthetised animals, the pancreas and the small intestine were dissected, after which the pigs were euthanized using an overdose of the barbiturate solution. Weights and lengths of intestinal organs were registered.

For the pancreas, total protein was determined using the Lowry method (Lowry et al., 1951). Trypsin activity was measured according to Fritz et al. (1966) with a modification by Pierzynowski et al. (1990), and amylase activity was measured using Phadebas Amylase Test (Pharmacia, Uppsala, Sweden). Sections (cranial, middle and distal) of the small intestine were analysed for intestinal disaccharidases (maltase, sucrase and lactase) according to Dahlqvist (1984).
Statistical Analyses

All statistical calculations were performed using the SAS System for Windows. Individual measures were used in paper I, whereas pen mean values were used in papers II-IV. The data was tested for normality with the procedure UNIVARIATE. Normally distributed parameters were thereafter analysed using the GLM procedure. Parameters that were found to be not normally distributed were tested with the 1-sided Wilcoxon 2-sample test. Logistic regression was used for analysing bacteriology data (I, II).
Results

Medical Treatments and Mortality

Out of the 48 + 62 piglets used for the PHA studies (I, II), 30 had to be treated with antibiotic injections due to signs of PWD (12, or 24%, of the PHA-treated and 18, or 37.5%, of the control pigs), and 7 pigs died (1 PHA-treated and 6 control pigs). In papers III and IV, of the 280 + 157 pigs, 78 pigs were treated with antibiotics (34, or 15.6%, of the trough fed and 44, or 20%, of the feed dispenser fed; 17, or 21.3%, of the mixed/moved and 1, or 1.3%, of the non-mixed/non-moved), and 6 died (3 trough fed and 3 feed dispenser fed; 1 mixed/moved and 1 not-mixed/not-moved).

Effects of Phytohaemagglutinin Exposure

Performance and Health

During the treatment week before weaning, the control pigs had a higher BW gain than the PHA-treated pigs (I, II), while in the first week after weaning, the PHA-treated pigs grew faster than the control (α-lactalbumin-exposed) pigs (I). From day 7 after weaning, there was no difference in BW gain between treatment groups.

The PHA-treated pigs tended to have a lower total diarrhoea score, and scores on a few individual days were significantly lower for the PHA pigs. The proportion of haemolytic E. coli was not statistically different between the treatment groups, but was numerically lower for the PHA-treated pigs.
Feed Intake Characteristics

On day 5 after weaning, the PHA-treated pigs spent more time eating than control pigs (I). Examination of the diurnal distribution of eating time, revealed that the differences in favour of PHA-treatment were most predominant during the night. Feeding bouts tended to be longer for the PHA-treated pigs on day 1 after weaning, and were significantly more numerous on day 5 after weaning (I).

No differences between treatment groups could be found with respect to feed consumption or FCR, except for a tendency towards greater feed consumption for the PHA-treated pigs on day 3 after weaning, and a numerically greater intake for the entire 2 week experimental period (II).

Physiological Parameters

The PHA-treated pigs had a lower uptake of both marker molecules into plasma (I) in comparison to that of the control animals. No differences between the treatment groups were found regarding the concentration of total protein or IgG in the plasma, although there was a greater increase in the plasma concentration of IgG between days 7-14 observed for the PHA-treated pigs (II).

The small intestine of the PHA-treated pigs tended to be longer, measured at 7 days after PHA exposure, than that of the controls. The enzymatic profile differed between treatment groups, with a lower lactase activity and higher maltase and sucrase activities in PHA-treated pigs. No differences were found with respect to the size or enzymatic content of the pancreas.

Effects of Weaning Strategies

Performance and Health

The BW gain of the mixed and moved pigs was lower than that of the not mixed and not moved animals. The small pigs were most negatively affected by the mixing and moving treatment (IV). To some extent, the BW gain was also impaired in pigs fed from feed dispensers in comparison to troughs (IV, not in III).

The small and large pigs had lower diarrhoea scores when being fed from a trough than from a feed dispenser. The opposite was found for the medium sized pigs. Small pigs had higher diarrhoea scores when being mixed and moved, than when they stayed with their littermates in the farrowing pen.
Feed Intake Characteristics

Not-mixed/not-moved pigs consumed more feed than the mixed/moved pigs did. The mixing procedure did not affect the overall FCR, whereas pigs fed from troughs had a higher FCR than did pigs fed from feed dispensers.

Small and large pigs fed from troughs spent more time eating (small pigs on day 1, and large pigs on day 5 after weaning), compared to being fed from feed dispensers. Trough fed pigs also had longer feeding bouts than feed dispenser fed (III, all size categories; IV, small pigs; in paper IV, medium pigs fed from feed dispensers had longer feeding bouts). Mixed and moved pigs (large on day 1 and medium day 5 after weaning) had longer feeding bouts than not-mixed/not-moved pigs.
Discussion

Phytohaemagglutinin Exposure

Hypothesis

It was hypothesized that the treatment of pigs with a crude preparation of PHA for 3 days during the week before weaning would induce maturation of the gut function, prevent the commonly observed growth check after weaning and reduce the occurrence of PWD.

Performance and Health

The PHA-treated pigs were initially, to some extent, affected by the treatment, because they grew slower than control pigs did during the week before weaning (the week of treatment). This effect has previously been noted in pigs (Rådberg et al., 2001) and rats (Linderoth et al., 2005a), and was considered to be an induction of temporary gut inflammation as a result of the lectin exposure (Linderoth et al., 2005a). The control pigs were given α-lactalbumin, which was chosen as control substance instead of saline because it was thought to be more relevant to use a protein in comparison to the tested lectin PHA. α-lactalbumin is a milk protein, already present in sow milk, but there is a possibility that added α-lactalbumin might be considered as a nutrient surplus, and thus be a reason for the improved growth of the control pigs. Although, with this taken into account, the fact still remains that in the present experiments; PHA-treated pigs had lower diarrhoea scores, and thus were more resistant to E. coli infection.

In paper I, it was shown that PHA-treated pigs grew faster during the first week after weaning, whereas a growth check was observed during the same period in the control pigs. This observation led to the studies reported
in paper II. Here, the pigs were housed in smaller pens with only 2 pigs per pen (as compared to 8 pigs per pen in paper I), belonging to the same treatment group, which should make it possible to determine whether the difference in BW gain was due to a difference in actual feed intake, or if the PHA treatment resulted in a more efficient FCR. Unfortunately, the difference in BW gain observed in paper I, was not repeated in paper II. This might have been due to differences in the experimental setup, with only 2 pigs per pen, which may have affected behaviour such as feed intake. The number of observations may also have been too small, because a few pigs died from PWD, which meant that both pigs had to be removed from the data material. With larger groups per pen, it would not have had as great an impact if one pig was taken out, but it would have been more difficult to determine individual feed intake.

It should be emphasized that the experiments were undertaken under conditions similar to those of a conventional pig farm, where there was normal infection pressure in order to determine the level of PWD as a result of PHA treatment. A different approach would have been to isolate the animals in a more experimental environment and use a challenge procedure, but then it would be more difficult to draw conclusions on the possible benefits under commercial production circumstances. Often studies on PWD are made on pigs experimentally challenged with pathogenic strains of *E. coli* (Melin et al., 2000; Nielsen and Szancer, 1994), but this does not necessarily induce diarrhoea (Melin et al., 2000). During the present experiment, to ensure that the diarrhoea outbreaks studied were *E. coli* associated, bacterial cultures from affected animals were sent to the Swedish National Veterinary Institute for analysis, which confirmed the presence of pathogenic *E. coli*, and in addition, post mortem examinations of all dead animals were carried out.

**Feeding Behaviour**

With respect to feeding behaviour, the differences between treatments were more obvious in paper I than in paper II, possibly for the same reasons as described above. The change of environment in paper II may also have affected the pigs’ behaviour. Although based on the results from paper I, showing longer feeding times and more feeding bouts for the PHA-treated pigs than for the controls, it can be concluded that PHA did have a beneficial effect on feeding behaviour, since it is of great importance that piglets keep eating after weaning to ensure that the nutritional requirements are met (Pluske et al., 1996). Dividing the total time spent feeding on more bouts may also enable the pigs to better utilise their feed-degrading capacity.
Maturation

In the small intestine, many morphological and functional changes take place at weaning. The height of the villi decreases, the crypt depth increases (see review by Pluske et al., 1997), and the enzyme profile changes with a decrease in the activity of the brush-border enzyme lactase and increase in the activities of maltase and sucrase (Aumaitre and Corring, 1978). Weaning stimulates pancreatic development and its enzymatic output, but there is a delay until the different enzymes reach efficient levels (Rantzer et al., 1997; Makkink et al., 1994; Cera et al., 1990). Thus, when the nutritional source is abruptly changed, the pig’s GI tract is not able to either fully digest the macronutrients from the feed or absorb all of what has been digested. This leads to an accumulation of undigested and unabsorbed feed which constitutes a media for growth of haemolytic E. coli bacteria in the GI tract (Palmer and Hulland, 1965; Kenworthy and Crabb, 1963), causing PWD.

In the present study (I), exposing the small intestine of the piglet pre-weaning to a red kidney bean lectin induced a similar functional development with respect to the small intestinal brush-border enzymes, measurable even on the day of weaning. This could imply that the feed can be more efficiently degraded by the digestive enzyme system, and that lower amounts of undigested feed will be accumulated in the GI-tract. If so, this could be the reason why fewer of the PHA-treated pigs developed PWD.

Previous studies have shown that PHA-treatment of suckling rats (Linderoth et al., 2005a,b) resulted in growth of the gut and pancreas. Except for a tendency to a longer total intestinal length in the PHA-treated pigs, no organ growth effects were observed in the present study (I). In contrast to other studies (Linderoth et al., 2005a,b; Grant et al., 1999) which showed a PHA-induced effect on the pancreatic output of digestive enzymes, no effect was observed on either the pancreatic activities of amylase or trypsin, or on the size of the pancreas itself. This might also partly have been due to the small number of pigs studied and the large variations between pigs of this age kept under conventional conditions.

In a previous study on suckling 14 d old piglets, a decreased marker molecule absorption into blood after gavage treatment with PHA had been observed, which indicated that the barrier properties of the intestine had increased due to exposure to the crude kidney bean lectin (Rådberg et al., 2001). In the present study, the observed reduction in the absorption of the inert and passively transported small marker, NaF, could be explained by a decrease in the intestinal surface area and fluid absorption. A decrease in the endocytotic capacity of the enterocytes might explain the reduction in BSA absorption after lectin treatment. Taken together, these results indicated that
a more efficient intestinal barrier function at weaning after lectin treatment had been achieved. In the study by Rådberg et al. (2001), morphometric analyses of the small intestine in lectin-treated pigs showed a decrease in villi heights, an increase in crypt depths and crypt cell mitotic indices, and decreased vacuolisation of the enterocytes in the distal small intestine, all indicating maturational changes. However, that study had been made on 10 day old pigs which had been sacrificed and examined only 2 days after PHA exposure, whereas in the present experiment pigs were approx. 4 weeks of age when PHA fed, and were sacrificed 5 days thereafter. This would imply differences in possible observations, such as a probable villi recovery from treatment, because the cell turnover has completed at least one cycle by the time of sampling. The change in the disaccharidase profile observed in the present study (I) was also an indication of maturation.

**Immunology**

Because PHA is an effective T-cell stimulator (Feldmann, 1998) and B-cells interact with T-cells to produce antibodies during antigen exposure (Porter, 1986), it has been assumed that PHA-treatment may affect the immune system and the IgG concentrations in the blood of the treated pigs. PHA is also a powerful oral immunogen that induces a high titer of anti-PHA antibody of the IgG isotype (Pusztai, 1993). While no differences in total plasma IgG level between the 2 treatment groups could be detected (I, II), it might have been useful to also analyze for specific anti-PHA IgG antibodies. The increase in the IgG levels observed after weaning in both treatment groups must be considered to be an effect of weaning and a response to the newly introduced feed antigens. The greater increase in the concentration of plasma IgG in the PHA treated animals, compared to that of the control pigs (II) might be interpreted as being due to an improved immune system and antibody synthesis, as a result of PHA treatment.

**Weaning Strategies**

**Hypotheses and Extended Theoretical Background**

The experiment in paper III was designed to maximise weaning stress, possibly leading to an increase in the incidence of PWD and other negative consequences of weaning, in order to be able to detect possible differences between feeding systems (feed dispenser or trough). The hypothesis was that the different feeding systems would affect feeding behaviour, and that the trough fed pigs would be able to eat whenever they wanted, regardless of
their relative size in the group. Mixing pigs at weaning is a common management procedure in many herds. From an experimental point of view it had the advantage of increasing stress and removing the effect of litter/sow. Infections present in some litters prior to weaning would not remain isolated in a specific farrowing pen, creating large differences between litters, but would be spread to more groups. In the study reported in paper IV, the aim was to investigate to what extent the mixing procedure itself constituted a stressor of importance, and if different size categories of pigs were equally affected by this management routine. In commercial pig production, the mixing procedure is normally performed to achieve some form of homogenous groups of specific number, according to sex or size. In this experiment, however, piglets were mixed into heterogeneous groups in order to form the same group structure as in the not-mixed litters.

Up until now, few studies have been conducted into how pigs of different relative size in a group react to weaning. It must, however, be mentioned that the same weaning weight in different individuals can have different causes, and thereby different prerequisites. One pig can have a specific weaning weight as a result of a larger milk intake, while another pig in the same litter may have achieved the same weight from consuming and successfully digesting creep feed. Naturally these two animals will not cope with weaning stress equally.

Feed dispensers having only a few eating places enable the designing of pens to be more flexible, whereas a trough requires a certain length along one side of the pen. The trough, however, provides the opportunity for all the pigs to eat at the same time, which can be of extra importance just after weaning, when the pigs’ ability to find their own feed is crucial, and feed intake is strongly influenced by social facilitation (Hsia and Wood-Gush, 1983). During the suckling period, all pigs in the litter suckle at the same time, and the total nutrient intake is divided into several meals over the entire day. Providing feed in troughs, preferably on more than one occasion during the day, would most likely make the transition easier just after weaning; however, this would require specific equipment or a larger amount of labour (Rantzer et al., 2004), affecting profitability. Trough feeding also enables restrictive feeding, which can be an efficient method of preventing PWD (Rantzer et al., 1996), and makes it easier to have a visual overview of all pigs in a pen.

Remaining with the litter in the farrowing pen after weaning by removing the sow reduces social stress for the individual pig, as compared with being mixed into a new group and moved at the same time. The mixed piglets do, however, have the advantage of being moved into a clean
pen at weaning, while the not mixed stay under the same bacterial pressure as before weaning.

**Body Weight Gain and Feed Intake Characteristics**

Mixing/moving at weaning affected the piglets’ BW gain more than feeding system did (IV). This meant that being regrouped was a greater stressor than having to learn a new way of obtaining feed. The small pigs were the most negatively affected in terms of BW gain as a result of both the mixing/moving treatment, and receiving feed from a feed dispenser. Feed consumption was significantly higher during the entire 2-week-period, for piglets reared as littermates. This could partly be explained by the fact that mixed and moved piglets spend more time on aggressive interactions when they meet their new pen mates. Previous studies (McGlone, 1986) of mixed weaned piglets showed that this aggressive behaviour was most frequent during the first hours, and markedly reduced after the first day after weaning. However, the present study showed that the mixing and moving procedure had more long-lasting effects.

With consideration to the entire 2-week experimental period in paper IV, the trough fed pigs had a higher FCR than did pigs receiving feed from a feed dispenser. This was due to the lower BW gain of the trough fed pigs, since there was no difference in feed consumption for the animals in the 2 feeding systems. In addition, the measured amount of consumed feed included possible feed spillage, which normally would be greater from troughs (O’Connell et al., 2002; Botermans and Svendsen, 2000) and a probable cause of the differences in FCR. The mixed and moved pigs had a lower feed consumption than did pigs weaned as entire litters, which contradicted the observations of previous studies by Pluske and Williams (1996), showing that mixing pigs at weaning increased their feed consumption and hence their BW gain.

**Post Weaning Diarrhoea**

Botermans et al. (2000) showed in a study on growing-finishing pigs that the smaller pigs reacted to an increase in competition by changing their feeding behaviour. In paper III, this competition effect was expressed by the occurrence of more severe diarrhoea outbreaks in small pigs fed from feeders than from troughs. The causes for this effect were not obvious, but probably the increased stress level contributed to making the small intestine of the small pigs more sensitive and prone to diarrhoea. The observations presented in paper IV showed that, especially for small pigs, both being fed from a feed dispenser and being mixed and moved, resulted in a higher occurrence of
diarrhoea. The peak around day 7 was not as pronounced in either the not-mixed pigs or for pigs fed from a trough. Large pigs in mixed and moved groups tended to have higher diarrhoea scores on day 5 than did the not-mixed pigs. This indicated that the large pigs were also sensitive to changes, especially since they might be large at weaning due to a high intake of milk, making the dietary changes at weaning even more abrupt. To our knowledge, this is the first time effects on health, as a response to different weaning strategies (mixing/moving and feeding systems), have been investigated.

**Feeding Behaviour**

The use of simple and standard feeders and troughs did not enable the evaluation of individual feed intake, or feed intake per size category. Instead the animals were videotaped, and thereby valuable information about the distribution of the feeding behaviour during 24 h could be obtained. It was also the only possible method of studying individual feed intake characteristics, because transponder surveillance technique is not possible for trough fed pigs. Previous studies have shown that feeding behaviour evaluated from videotaped information was highly correlated to the actual feed intake (Fraser *et al.*, 1994; Appleby *et al.*, 1992), although it was a very time-demanding method if, as in this case, the registration process from the tapes was completely manual.

When evaluating feeding behaviour in the form of bouts, interesting results have been presented in paper III, showing that pigs fed from a trough were able to stay longer times at the trough at each feeding opportunity, and this probably provided a less stressful feeding environment.

Studies on growing-finishing pigs by Botermans *et al.* (2000) showed that access to a smaller feeding space resulted in the small pigs eating a larger proportion of the feed during the night, as compared to having more feeding places available per pen. The study in paper IV, however, found that small newly weaned pigs spent more time eating during the night when trough fed, than when fed from a feed dispenser, which disagreed with the observations of the previous studies. This might have been an indication that the small pigs needed other littermates around them, perhaps to take the initiative to start feeding, similar to the nursing situation of a few days previously. Work by Bruininx *et al.* (2001), has shown that the time between weaning and the first intake of feed varied greatly between individuals; taking up to 50 h before all the pigs in a group have started eating. It could be assumed that using feed dispensers, in comparison to
troughs, results in the pigs requiring more time to learn to find and operate them, since dispensers do not facilitate synchronous feeding.

**General Aspect of Weaning Problems**

A vast amount of research resources is being spent on trying to understand and overcome the problems caused by early weaning, which may appear to be a paradox, since the early weaning is a restriction placed on the animals by humans; a problem that we have created ourselves. Obviously, the solution appears to be to simply increase weaning age, but that is practically impossible, since the entire production system is built around sows nursing their litter for, in Sweden, 4–6 weeks. Production incomes would decrease with fewer weaned piglets per sow and year, and a different housing system would be required if the piglets were to be allowed to stay with the sow for a longer time period. Still, the fact that weaning problems are caused by the way we produce pigs, makes the amount of effort to overcome them – this thesis included – worth reflecting upon.
Conclusions

- Enteral exposure of PHA to pigs during 3 days the week before weaning resulted in more mature properties of the small intestine in the form of more efficient intestinal barrier functions and a change toward a more adult disaccharidase pattern.
- PHA-treated pigs had lower BW gain during the treatment week, but improved BW gain during the week after weaning, less severe PWD, and a feed intake pattern which better utilised the pigs’ digestive capacity.
- Trough feeding resulted in lower diarrhoea scores for the small and large pigs in a heterogeneously composed group with respect to body size, as compared to being fed from a feed dispenser.
- Trough feeding allowed the pigs to spend longer time eating per feeding bout, compared to being fed from a feed dispenser.
- Trough feeding imitates the nursing situation in that all pigs can eat at the same time, which is a preferred behaviour of pigs, but probably of extra importance just after weaning.
- Mixing and moving pigs at weaning resulted in a lower feed intake and lower BW gain in the first weeks after weaning, compared to being weaned as entire litters.
- Different relative body size categories of pigs reacted differently to the tested weaning strategies, which showed the importance of not just compare pen mean values when evaluating management routines.
Areas for Future Research

- PHA was chosen as a model substance for the initiation of gut maturation because of its known properties, but it is possible that other substances and administration methods can induce the same type of maturational changes. This remains to be further investigated in the future.
- The mechanisms by which PHA acts to affect BW gain, diarrhoea and feeding behaviour are largely unknown. Fields of interest would be to study the interaction of PHA with the immune system and with the intestinal microflora.
- Further research is needed to establish the causes for the disparity found in severity of PWD between feeding systems with different number of eating places.
- In order to measure individual feed intake of group housed pigs having the possibility of feeding at the same time, new methods and/or techniques need to be developed.
Sammanfattning på svenska

Avvänjning av smågrisar innebär att sugga skiljs från sina kultingar. Detta sker i Sverige när grisarna är 4-6 veckor gamla och väger ca 7-10 kg. Jämfört med hur grisarna skulle ha avvants i naturen, är detta en tidig avvänjning, som dessutom sker abrupt. Efter avvänjningen måste små-grisarna, som är vana vid att dia, lära sig äta en ny typ av foder. Ett vanligt problem som uppstår är att smågrisarna får s.k. avvänjningsdiarré omkring en vecka efter avvänjning. De drabbade grisarna kan dö av uttorkning, om inte sjukdomen snabbt hävs genom antibiotikabehandling. Diarrén orsakas av patogena *E. coli*-bakterier, som just kring avvänjnngen har möjlighet att fästa på tarmslumhinnan. Grisarna har dålig immunstatus pga. att de antikroppar de fått i sig via råmjölken, och som sedan tas upp i blodet, har börjat avta, och den egna antikroppsproduktionen är ännu inte tillräckligt effektiv. Suggmjölken innehåller också antikroppar som verkar lokalt i tarmen, men efter avvänjningen finns inte detta skydd kvar. Vidare påverkar stressen i samband med avvänjningen, immunförsvarset ytterligare i negativ riktning. Det tar några dagar för grisarna att komma igång att hitta och äta sitt nya torrfoder, och dessutom är inte matspjälningssystemet förberett på att smälta annat än mjölk. Detta gör att fodret stannar osmält i mag-tarmsystemet under tillräckligt lång tid för att *E. coli*-bakterierna ska börja växa till, med fodret som näringskälla.

I denna avhandling undersökt om det med hjälp av lektinet PHA (phytohaemagglutininen) var möjligt att påbörja en mognadsprocess i tunntarmen redan före avvänjning, för att på så sätt göra grisen fysiologiskt bättre förberedd för avvänjningen. PHA utvinns ur röda kidneybönor, och är sedan tidigare känt för att ha en tillväxtstimulerande effekt på mag-tarmsystemet. En vecka före avvänjning gavs PHA med magsond till grisarna under tre på varandra följande dagar. Under veckorna närmast efter avvänjningen studerades grisarnas tillväxt, diarréförekomst och haemolytisk
E. coli-förekomst i avföringen, genom upprepade mätningar och analyser. Grisarnas ätbeteende registrerades, och blodprov togs för att mäta i hur hög utsträckning tarmen var genomsnittlig för markörmolekyler av olika storlek, samt för att mäta grisarnas immunstatus. Några grisar avlivades på avvänjningsdagen, varefter deras matspjälkningsorgan togs ut och vägdes/mättes. Prover togs från bukspottkörteln och tunntarmen, för att undersöka om grisarna hade fått en annan, mognare enzymprofil, som en följd av PHA-behandlingen.

Resultaten från PHA-försöken visade att grisarnas tillväxt kring avvänjningen påverkades av behandlingen. Under behandlingsveckan före avvänjning växte kontrollgrisarna bättre än PHA-grisarna, och i en av två delstudier hade PHA-grisarna en högre tillväxt än kontrollerna under första veckan efter avvänjning. PHA-grisarna fick inte diarré i lika hög utsträckning, och ägnade mer tid åt att äta, samt delade upp sitt ätbeteende på fler tillfällen under dygnet. De fysiologiska effekter som uppättes hos PHA-behandlade grisar var ett lägre markörmolekylupptag från tarmen till blodet, och en annorlunda profil av kolhydratspjälkande enzymer från tunntarmen; båda dessa är tecken på en mer mogen tunntarm.

Foder kan ges i olika typer av automater och tråg till nyavvanda grisar. I denna avhandling studerades hur grisar av olika relativ storlek (små, mellan och stora) i grupper med 10 grisar reagerade på att antingen bli utfodrade i ett tråg där alla kunde äta samtidigt, eller från en friutfodringsautomat med 2 ätplatser. Tillväxt, diarréförekomst och ätbeteende registrerades, och det kunde konstateras att i synnerhet de små, men även de stora grisarna fick mindre diarré vid trågutfodring. De mellanstora grisarna hade mindre diarré vid automatutfodring. Alla storlekskategorierna tillbringade längre tid vid tråget än vid automaten vid varje ättillfälle.

Det studerades också hur samma storlekskategorier av grisar reagerade på att bli blandade till nya grupper som flyttades till en ny box vid avvänjning, jämfört med att stanna i sin kull och box. Grisarnas foderintag och tillväxt var lägre i blandade grupper, och i synnerhet de små grisarna växte sämre om de blev blandade och flyttade. Små grisar fick också mer diarré om de blev blandade.

Sammanfattningsvis kan det konstateras att PHA-behandling måste anses som en intressant möjlighet för att komma tillrätta med avvänjningsproblemen. Blandning av grisar vid avvänjning, som är vanligt i kommersiell produktion, utgör en stor stressfaktor för djuren, och genom att undvika det kan grisarna få en bättre tillväxt efter avvänjningen. Direkt efter avvänjning bör grisarna ges möjlighet att äta foder från ett tråg, eftersom alla då kan äta på samma gång, dvs. på samma sätt som de är vana vid sedan di-tiden.
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