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Feed intake, nutrient digestibility, growth performance and general health of piglets fed increasing levels of yeast

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Summary

In Norway, the climatic conditions and limited agricultural area impedes production of protein-rich plant-feedstuff, leading to the current dependency of import of soybeans. Norway has large forest areas, which constitute a huge lignocellulosic biomass with unused potential as resources for production of microbial feed ingredients with high-protein content such as yeast. Studies have also shown that yeast or other bioactive compounds may help the piglets to better cope with challenges due to weaning and transition of feed, by providing beneficial effects on the intestinal health. The aim of this thesis was to evaluate the effect of increasing dietary level of *Candida utilis* yeast biomass on nutritional value and general health responses in early weaned piglets over a period of four weeks post-weaning. A total of 48 piglets, weaned at four-weeks of age, were distributed to four dietary treatments; a control feed (0 % yeast) and three diets containing increasing amounts of yeast corresponding to 10 % (Yeast 10), 20 % (Yeast 20) and 40 % (Yeast 40) of total CP in the diet. Body weight gain and feed intake were registered weekly. From day 18 to 28, the piglets received feed labeled with Y_2O_3 as digestibility marker. Feces were collected in a period of five days for determination of apparent total tract digestibility (ATTD) of nutrients, and ileal content were collected at the termination day for determination of apparent ileal digestibility (AID) of nutrients. The first week PW there was a linear increase in average daily gain (ADG) and fecal DM by increasing levels of yeast, indicating the yeast may improve intestinal health of weanling piglets. In addition, for the four-week period, average fecal consistency scores were significantly lower piglets fed Yeast 40 compared to the control. Digestibility of most nutrients and feed utilization of the diets containing yeast was similar to the control, except for the Yeast 20 diet. Digestibility results showed an improved ATTD of ash in the piglets fed Yeast 10 and Yeast 40. In conclusion, the results of this study indicate that *Candida utilis* yeast produced from lignocellulosic biomass may be a suitable protein source in feed for weanling piglets.

Sammendrag

Klimatiske forhold og begrensede områder med dyrkbart areal, vanskeliggjør produksjonen av proteinrike plantefôrråvarer i Norge. Dette gjør at vi i dag er avhengige av import av soyabønner som proteiningrediens i kraftfôr. Norge har imidlertid store skogområder, som med ny teknologi kan utnyttes til produksjon av proteinrike mikroorganismer, slik som gjær. Det er også vist at gjær eller bruk av lignende bioaktive komponenter i fôret kan ha positiv innvirkning på tarmhelsen hos grisunger under avvenning. Hensikten med denne oppgaven var å evaluere næringsverdi og helserespons hos tidlig avvente smågris, ved økende nivåer av gjæren *Candida utilis* i fôret, i de første fire ukene etter avvenning. 48 griser avvent ved fire ukers alder ble fordelt på fire ulike dietter; et kontrollfôr (0 % gjær) og tre dietter med økende innhold av gjær tilsvarende 10 % (Yeast 10), 20 % (Yeast 20) og 40 % (Yeast 40) av totalt råproteininnhold i diettene. Fôrinntak og vekt ble registret ukentlig, og fra dag 18 til 28 i forsøksperioden var fôret tilsatt markør (Y_2O_3) for bestemmelse av fordøyelighet. Det ble samlet gjødsel over en periode på fem dager for bestemmelse av apparent totalfordøyelighet (ATTD) av næringsstoffer, og tarminnhold fra ileum ble samlet avslutningsvis for bestemmelse av apparent ileal fordøyelighet (AID) av næringsstoffer. Den første uken etter avvenning var det en lineær økning i gjennomsnittlig daglig vekst (ADG) og tørrstoff i gjødsel, ved økende innhold av gjær i fôret. Dette indikerer at gjæren kan bedre tarmhelsen under avvenning. Gjennomsnittlig gjødselscore for forsøksperioden var signifikant lavere hos dyrene som fikk Yeast 40 dietten sammenlignet med de som fikk kontrollfôret. Fordøyeligheten av de fleste næringsstoffene og fôrutnyttelse av diettene som inneholdt gjær var sammenlignbart som for kontrolldietten, bortsett fra for Yeast 20 dietten. Fordøyelighetsresultatene viste forbedret ATTD av aske hos griser som fikk Yeast 10 og Yeast 40 diettene. Som konklusjon viser resultatene i forsøket at *Candida utilis* gjær fremstilt fra lignocellulosisk biomasse kan være en egnet proteinkilde i fôr til avvente smågris.

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List of abbreviations

AA = amino acids

ADG = average daily gain

ADFI = average daily feed intake

AGP = antibiotic growth promoters

AID = apparent ileal digestibility

ATTD = apparent total tract digestibility

BPM = bacterial protein meal

BW = body weigh

CF = crude fat

CP = crude protein

DM = dry matter

FCR = feed conversion ratio

GE = gross energy

GIT = gastrointestinal tract

OM = organic matter

PUFA = polyunsaturated fatty acids

PW = post-weaning

SBM = soybean meal

SCP = single cell protein

SID = standardized ileal digestibility

TID = true ileal digestibility

1.0 Introduction

In 2015, 40 % of consumed meat in Norway was pork (Alvseike et al. 2016). An efficient pork production require feed of high quality that covers the nutritional needs of the animals. Currently, the Norwegian pig production is dependent on import of soybeans from Brazil as a protein source in the feed. Soybeans has a high protein content, well complementary to the Norwegian grains. The proportion of Norwegian protein commodities in livestock feed has decreased from about 50 % in 1997 to about 5 % in 2015 (Landbruksdirektoratet 2015). This is mainly caused by the demand for high quality feed to cater increased production efficiency, both in the livestock and aquaculture industry, but also because of the ban on the use of meatbone meal (in 2003) and fishmeal (in 2010) (Forskrift om animalske proteiner i dyrefor 2007; Forskrift om TSE 2004). Although, use of fishmeal is allowed in pig and chicken feed (Glende 2014).

In Norway, the climatic conditions and limited agricultural area impedes production of protein rich plant feedstuff. Worldwide, the production and export of soybeans also has an ecological point of view as the production occupying huge areas, promoting deforestation of the rainforest. In the latest parliamentary report (Meld. St. 11 2016), from the Royal Norwegian Ministry of Agriculture and Food, emphasis is placed on a demanding international market in change, and a demand for increased production based on Norwegian resources. Developing feed ingredients from new resources is an important contribution to reach this goal. Additionally, the development of such feed ingredients is also important for self-sufficiency.

2.0 Literature review

2.1 Demand for new feed ingredients

In the last 50 years, the world population has more than doubled. An increasing world population also increases the need for food. However, the cultivated area is limited. Thus, not fully exploited, but the agricultural potential is not evenly distributed between countries. Different climatic conditions causing some countries having better conditions for food production than others. Therefore, trade between countries is crucial to supply the world population with food.

The increased demand for food and feed ingredients from a growing population, is leading to increasing rates on the world market. In addition, production and transportation costs has increased. A demanding market and world conflicts makes it even more important to base food production on local resources. There is also a need to exploit other resources for feed production, not occupying land area for human food production. For instance, residues from the food industry constitutes a major potential as feedstock in livestock production, with a low environmental impact (Elferink et al. 2008).

Livestock production represents a valuable protein source in the human diet, especially in Europe and Northern-America. Whereas the prosperity in the world increases, consequently the demand for meat increases in other parts of the world. However, livestock production demands large quantities of feed, thus constitute a large percentage of world trade. Producing plant feed ingredients occupies huge areas suitable for human food production. Often, the production could go to human consumption instead of feed ingredients.

The production of feed should not compete directly with human food production. This is both an economical and ethical question, as the food shortages in the world is critical. Instead, livestock production should be used as an efficient way to upgrade low quality (non-human food) material (such as microalgae, bio-waste, grass and trees) into high quality foods (Gabriel et al. 2014; Nasseri et al. 2011). Innovations can make it possible to exploit biodegradable waste to produce high quality protein feed ingredients. Research is done continuously in this area, and microbes such as yeast and bacterial protein meal (BPM) (Anupama & Ravindra 2000), but also insects, is seen as future protein sources (Veldkamp et al. 2012).

Yeast and bacteria have been used for centuries in food production. However, mainly not as protein source, but for other advantages in production; fermented yeast for bread, beer and wine, and lactic acid bacteria in yoghurt and cheese. Throughout history, periods of protein deficiency has created focus on use of these organisms as new protein sources (Kuhad et al. 1997; Upadhyaya et al. 2016).

2.1.1 Single cell protein

In 1966, the term "single cell protein" (SCP) was coined, by professor Carroll Wilson, as a collective term to replace less aesthetic terms used (Snyder 1970). Two years later, Mateles and Tannenbaum (1968) publish an article claiming that within few years, SCP will have a significant impact on the nutrition of people.

The term covers protein produced from bacteria, yeasts, microscopic fungi and microscopic algae. In general, SCP has high protein value and are therefore a well suitable protein source in feed (Øverland et al. 2010; Øverland & Skrede 2016). Additionally, the lysine content of SCP in general is high, in contrast to many plant proteins (Mateles & Tannenbaum 1968). However, the quantities of individual amino acids in microorganisms are affected by growth stage, aeration and age of the cell (Stokes & Gunness 1946). Moreover, a challenge due to including microorganisms in feed, may be their lower digestibility and possibilities for allergic reactions (Nasseri et al. 2011). Therefore, research is needed on the specific SCP source and on the use in the feed for that particular species.

A number of factors must be taken into consideration when choosing microorganism for SCP production; the nutritional value (protein content, amino acid (AA) content, digestibility), economical (cost of substrate and nutrients), production technological (production methods and efficiency) and feed technological factors for making the diet (Mateles & Tannenbaum 1968). Advantageous features with the SCP production includes wide range of microorganism species, methods and raw material for growth. Generally, microorganisms have a high efficiency in substrate conversion and a fast growth rate, resulting in high productivity. Additionally, SCP production is independent of seasonal factors and production does not require large quantities of fresh water or agricultural land areas (Nasseri et al. 2011).

Table 2.1 Average composition of microorganisms, % of dry weight (Gabriel et al. 2014).

	Microorganism		
Nutrient	Bacteria	Microalgae	Yeasts
Protein	50-65	40-60	45-55
Fat	1.5-3.0	7-20	2-6
Ash	3-7	8-10	5-9.5
Nucleic acids	8-12	3-8	6-12

For human consumption, the high nucleic acid content in SCP is a challenge (Nasseri et al. 2011). Degradation of nucleic acids in the body leads to production of uric acid, which can accumulate in the body (Anupama & Ravindra 2000). However, whereas this uric acid accumulation can lead to gout or development of kidney stones in the urinary system in humans, most animals (including pigs) possess the enzyme urate oxidase (Wu et al. 1989). This enzyme allows them to transform uric acid into allantoin, which is easily excreted in the urine (Andersen et al. 2006; Wu et al. 1989).

When new feed ingredients are being produced, feed safety is always an issue. Some microorganism produces toxic compounds and may be pathogenic. Knowledge of the organism, such as produced metabolites and residues from the fermentation process is important information (Kuhad et al. 1997). During production, hygiene is important to avoid contamination by pathogenic microorganisms, which often have a high growth rate.

2.1.2 Bacterial biomass in feed

Bacteria has the highest growth rate of the microorganisms (Kuhad et al. 1997) and a high biomass yield, as well as a high protein content (Kuhad et al. 1997; Upadhyaya et al. 2016). Average composition is shown in Table 2.1. However, due to their small cell size, production and separation is comparatively energy demanding, and production profitability depends on the availability of inexpensive substrate (Kuhad et al. 1997).

Protein synthesis through bacterial fermentation from methanol was a major biotechnological breakthrough in the 1970s (Upadhyaya et al. 2016). Later, methane has been used to produce BPM as a protein feed ingredient (Øverland et al. 2001). BPM from the methane utilizing bacteria (*Methylococcus capsulatus*), have been shown to be an excellent feed ingredient to both monogastric animals and farmed fish (Øverland et al. 2010). However, some bacteria are pathogenic and therefore an intensive screening of the strain is needed before it can be included as a feed ingredient (Upadhyaya et al. 2016).

2.1.3 Microalgae in feed

Microalgae biomass is suitable as a protein source comparable to conventional vegetable proteins (Becker 2007). A high lysine content makes microalgae well suitable in cereal diets (Kuhad et al. 1997). Microalgae may contain high amounts of polyunsaturated fatty acids (PUFA), including the omega-3 fatty acids; docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), and the omega-6 fatty acids; arachidonic acid and γ-linoleic acid. Micoralgae may therefore also be cultivated as a lipid-feedstock (Lum et al. 2013). The unicellular and filamentous blue-green microalgae *Spirullina* is known for a high content of protein, essential amino acids and PUFAs, and has been revived and used in human, animal and aquaculture nutrition. However, due to the non-digestible cell wall, proper processing is needed before the microalgae biomass can be used in feed for non-ruminants (Becker 2007).

Advantage of microalgae is their ability to grow with carbon dioxide as the only carbon source. Growth of microalgae can take place using sunlight or artificial light, photosynthetically and autotrophically, but also heterotrophically without light sources (Kuhad et al. 1997). Using wastewater from factories, growing of the microalgae may both be economical and environmentally beneficial (Upadhyaya et al. 2016). The de-fatted microalgae biomass from biofuel production, may also be exploited and used as an animal feed ingredients (Lum et al. 2013).

2.1.4 Yeast in feed

Yeasts are eukaryote unicellular organisms with a high nutritional value. Compared to bacteria, yeasts has a lower toxic potential and is therefore more widely accepted (Kuhad et al. 1997). Yeast also have a lower nucleic acid content than bacteria (Kuhad et al. 1997; Stringer 1982), and is a good source of vitamin B. By living inclusion, yeast may act as a probiotic, which are live feed ingredients that may improve the host animal's intestinal microbial balance (Fuller, 1999).

The yeast species *Saccharomyces cerevisiae* have been used in fermentation of foods for centuries (Legras et al. 2007), and is commonly accepted as an additive in food and feed. Research on other strains are done the last decades, exploring the possibility to use yeast as a protein source (Spark et al. 2005; Øverland et al. 2013; Øverland & Skrede 2016).

Already in 1957, Thaysen compared protein production efficiency between beef animal, soybean and yeast, showing that yeast have a huge potential in protein production as kg per day. Since then, several studies has been done about the effect of and resources in yeast (SCP) (Kuhad et al. 1997), but due to the question of cost, the production has never expanded to the feed industry.

2.1.5 Lignocellulosic biomass as raw material

In Norway, about 40 % of the area is forest and only about 3 % is cropland (*Norge - Arealbruk og jordbruk* 2015). The forest constitute a huge lignocellulosic biomass with unused potential as resources for production of feed ingredients. Lignocellulose consist of lignin, hemicellulose and cellulose, constituting the major component of all plant biomass (Gabriel et al. 2014). Through forestry and agricultural industries, large amounts of lignocellulose is generated as waste. Innovations have made it possible to exploit lignocellulosic biomass and biodegradable waste as sources for renewable organic matter, including production of biofuels, chemicals, energy sources for fermentation, microbial bioconversion into SCP, animal feed, and human nutrients (Howard et al. 2003).

There are different pretreating methods to increase the availability of degradable carbohydrates in the biomass. By removing lignin and increase the release of cellulose and hemicellulose sugars, lignocelluloses become a resource for organisms which are not able to produce necessary enzymes for degradation (Gabriel et al. 2014). The major steps in processing of lignocellulosic biomass for yeast production are 1: pre-treatment, 2: enzymatic hydrolysis, 3: fermentation, and 4: downstream processing (Figure 2.1; Øverland & Skrede 2016).

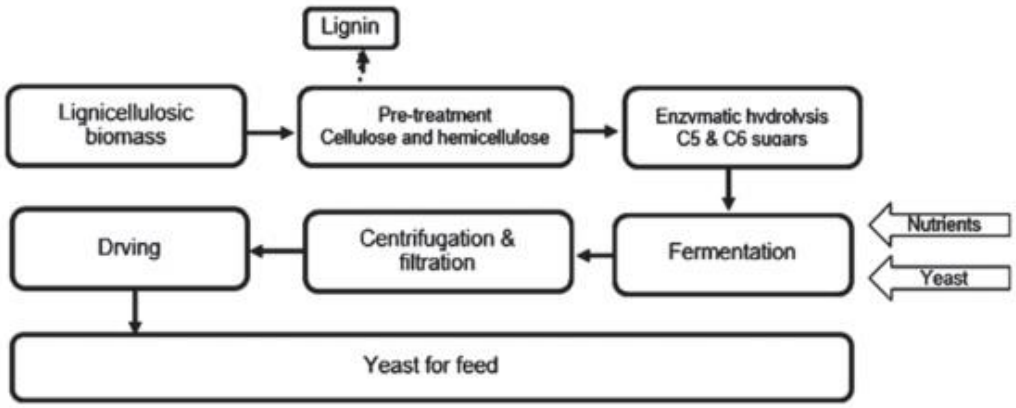


Figure 2.1 Steps in the processing of lignocellulosic biomass for production of yeast (Øverland & Skrede 2016).

2.2 Digestibility in monogastric animals

Pigs are monogastric animals, and their digestive system is very similar to humans. Gross anatomy are shown in Figure 2.2. The digestion of the feed mainly occurs in the small intestine, where digestive enzymes are secreted and the nutrients are degraded and absorbed over the intestinal epithelium. Segmentation, churning and mixing, helps the dietary molecules to come in contact with secreted enzymes, and increases the intestinal contents contact with the intestinal surface, thereby enhances degradation and absorption of the nutrient. After digestion, peristaltic waves transport the content throughout the intestine (McDonald et al. 2011). Undigested and unabsorbed chyme reaching the colon may be fermented by the microbiota, producing volatile fatty acids (VFA), which is absorbed over the intestinal wall and used as an energy source for the animal. In addition, microbiota in the hindgut are synthesizing large amounts of protein, and may produce some vitamins. However, a lack of proteolytic enzymes and a transport system in the hindgut prevents absorption of these nutrients (McDonald et al. 2011).

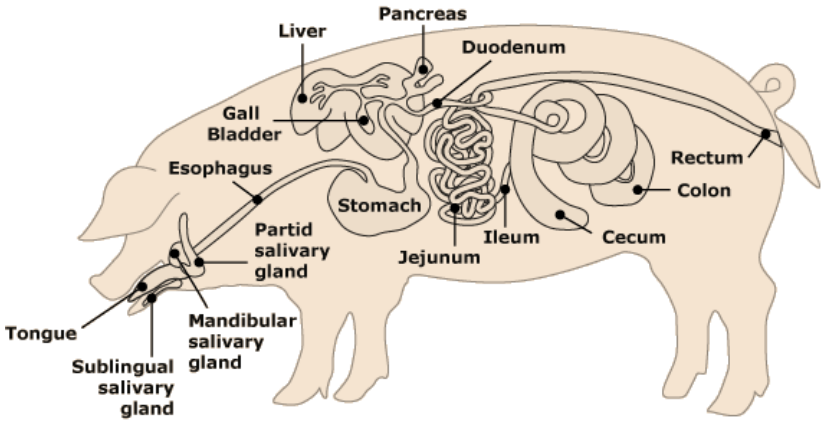


Figure 2.2 Digestive anatomy of the pig (French 2004).

The digestibility of a feedstuff depends on its chemical composition, but is also influenced by the mixture of feedstuffs in the diet, and the processing during feed production (McDonald et al. 2011). Monogastric animals lack some appropriate enzyme systems for utilization of some of the nutrients in the feed. Feed processing methods and enzyme preparations may thus increase nutrient availability. Moreover, anti-nutritional factors in different ingredients can bind to proteins and AA, which may reduce their digestibility (McDonald et al. 2011). In Norway, pig feed is usually pelleted. Pelleting is an energy-demanding process purposely to ensure a homogenous feed intake. During the conditioning and pelleting process, heat appliance affects the nutrients in the feed. Denaturation of proteins may improve nutritional value, whereas the process also may destroy some vitamins (Svihus & Zimonja 2011).

2.2.1 Methods for measuring of digestibility

There are various methods for measuring digestibility, including use of cannulas in the intestine or collection of feces. Sampling and analysis of feces determines total tract digestibility, while sampling of ileal digesta outflow determine ileal digestibility. Estimating the digestibility of nutrients in the diets require total collection of digesta or feces, or addition of an inert marker with a known concentration into the diet.

In order to measure the digestibility of feeds in successive sections of the digestive tract, it is most conveniently to use cannulated animals (McDonald et al. 2011). The mobile nylon bag method is a relatively rapid and inexpensive method to measure digestibility in pigs which in addition allows determination of digestibility in individual feed ingredients (Yin et al. 2002). The method involves insertion of small samples of food contained in nylon bags into the duodenum through a cannula, later recovered via a second cannula in the ileo-ceco-colic junction (Yin et al. 2002) or collected in the feces (Sauer et al. 1983). This method involves surgical implantation of one or more permanent cannulas. However, another option is collection of digesta in the intestine by slaughtering the animal. Comparing the methods, Pedersen et al. (2010), found no differences between the slaughter and T-cannulation methods on ileal digestibility of dry matter (DM), organic matter (OM), ash or crude protein (CP).

2.2.2 Use of a digestibility marker

A digestibility marker should meet the following requirement: 1. the marker should homogenously incorporate into the feed and be easily and accurately analyzed; 2. it must be indigestible and not affect the metabolism of the animal; 3. the marker should pass through the gastro-intestinal tract at the same rate as the dietary nutrients, and it should; 4. be hygienic and harmless to people and the environment (Austreng et al. 2000).

Chromium oxide (Cr_2O_3) is a commonly used inert marker in digestibility studies, but it has proven not to behave optimally (Austreng et al. 2000). However, Titanium dioxide (TiO_2) has been shown to be an appropriate marker in pigs (Jagger et al. 1992). In addition, use of Yttrium oxide (Y_2O_3) as an inert marker has been proven to give precise digestibility determination in dogs, blue foxes and mink (Vhile et al. 2007).

2.2.3 Protein digestibility

The profile of digestible essential AA is the most important single factor affecting the efficiency of protein utilization (Boisen et al. 2000). AA is only absorbed in proximal to distal ileum. A challenge of estimating protein digestibility is the endogenous supply in the intestine. The ileal digesta contain both unabsorbed exogenous AA of dietary origin and AA of endogenous origin, although most of the AA and small peptides released by the digestive enzymes usually are reabsorbed (Mosenthin et al. 2000). The AA of endogenous origin are consisting of two sections; the quantities of AA that will be lost from the animal regardless of the diet (basal IAA_{end}), and losses induced by specific feed ingredient characteristics (spesific IAA_{end}) (Stein et al. 2007).

In the cecum and colon, undigested components are in a large extent fermented by the microflora. The microorganisms may deaminate AA, using the carbon for energy. The ammonia then can be absorbed, converted to urea in the liver, and excreted in the urine. On the other hand, the microbiota may also commit net synthesis of some AA. Due to the microbial metabolism, digestibility values obtained by fecal analysis method may overestimate or underestimate the AA digestibility obtained by the ileal analysis method (Mosenthin et al. 2000). Consequently, in determination of AA absorbed by the pig, ileal analysis method rather than fecal analysis method is more accurate (Mosenthin et al. 2000; Tanksley et al. 1981).

Ileal digestibility of AA can be expressed as apparent (AID), true (TID) or standardized (SID) ileal digestibility depending of which proportion of the ileal AA outflow is included in the calculation (Stein et al. 2007). AID represent the net disappearance of AA from the digestive tract, calculated by subtracting the total ileal outflow from diet consumption. For calculating TID and SID, endogenous losses must be known or calculated. Basal endogenous losses are independent of the diet composition, but dependent of dry matter intake. Hess and Seve (1999) reported a linear effect of feeding level on basal endogenous losses (g/day) in growing pigs. Specific endogenous losses represent losses above the basal losses, induced by specific feed ingredient characteristics, such as the concentration and type of fiber and anti-nutritional factors (Stein et al. 2007).

Equations for calculating ileal digestibility. From (Stein et al. 2007).

AID (%) = [(AA intake – ileal AA outflow) / AA intake) * 100

TID (%) = [(AA intake – (ileal AA outflow – total IAA_{end})) / AA intake] * 100

SID (%) = [(AA intake – (ileal AA outflow – basal IAA_{end})) / AA intake] * 100

Since AID calculation may underestimate digestibility of low-protein sources (Boisen et al. 2000), calculation of SID should be used in diet formulation (Stein et al. 2007). However, calculation of SID requires an estimate of the amount of non-spesific endogenous protein and AA recoveries in ileal digesta (Mosenthin et al. 2000). Moreover, AID values obtained for individual feed ingredients are not additive in a mixed diet, whereas this applies to values for SID. However, values for AID may be used to estimate net absorption of AA from a specific diet (Stein 2006).

2.2.4 Digestibility of other nutrients

Starch, from cereals, is the main source of energy in diets for monogastric animals. Starch is a highly digestible carbohydrate, often assumed to be completely digested in the small intestine, but some starches may pass through and contribute as an energy source for the microbiota in the colon. Fermenting of the starch by the microbiota will lead to lower energy supply to the animal than if digested in the small intestine, and may cause an excessive large intestinal fermentation, leading to diarrhea (Wiseman 2006).

2.3 Challenges for weaned piglets

2.3.1 Weaning

The term weaning is used for the moment piglets are separated from their mother, and simultaneously needs to change their diet from milk to solid feed. In addition, weaning often involves new environment and mixing of litters. Hence, weaning represent a stress factor for the piglet. In Norway, it is stated by law that weaning of piglets may not occur before 28 days of age (Landbruksdepartementet 2003). Average age of weaning in Norway is 33.1 days of age (Ingris 2015). In nature, the weaning process occurs more gradually over a period, finished at about 14-16 weeks of age (Jensen 1986).

At weaning, the piglets are vulnerable to disease as they are going through major changes in diet and environment, causing increased level of stress. Often, weaning causes a growth decline for the piglet. Weight gain during first week post-weaning (PW) have major impact on subsequent growth performance (Kats et al. 1992), and is therefore of particular importance for the economy in the production.

In their review *"Understanding weaning distress"*, Weary et al. (2008) conclude it is the sum of change leading to increased stress for young mammals. Change in diet itself have little impact, if environment and social contact with mother remains unchanged.

2.3.2 Development of the digestive tract in the weanling piglet

The digestive system of newborn piglets is adapted to digest milk-intake in small frequent meals. As they grow older, the digestive system mature and develop for digestion of solid food. The major maturation of the digestive system occurs during the first two month of life (Manners 1976). Weaning constitutes a critical phase as it usually occurs before the digestive system of the piglet is fully developed. Thus, it is likely that this huge change in diet during the maturing phase may affect the development.

It is well established that weaning cause changes in gastrointestinal tract (GIT) structure and function. Several authors describe changes; in intestinal histology such as villous atrophy and crypt hyperplasia (Cera et al. 1988; Lallès et al. 2007b; Pluske et al. 1997), tongue and leaf-shaped villi (Cera et al. 1988; Makkink et al. 1994), increased mucosal permeability (Lallès et al. 2007a; Spreeuwenberg et al. 2001), changes in enzyme activity (Hampson & Kidder 1986; Lindemann et al. 1986; Makkink et al. 1994) and gut microbiota (Lallès et al. 2007b), due to weaning.

Kelly et al. (1991a) studied the effects of continuous nutrient supply on the development of the digestive tract during the first week (PW) and compared to sow-reared piglets. The piglets were fed by gastric incubation, providing an adequate nutrient supply to the GIT. Results inducted that histological changes in the initial weaning period occurs irrespective of sufficient nutrient supply. However, the nutrient level may influence the extent of these changes. In another study by Kelly et al. (1991b), two different feeding-strategies (restricted and continuously) for piglets weaned at 14 days of age was examined. Results revealed differences in anatomy, morphology and function of the GIT between the different treatments. These results suggesting GIT development may be affected by several factors. Although, there is a maturing process of the GIT that takes place independent of nutrient intake, there is also an adaptive part of the GIT development influenced by the amount of nutrient intake.

In their review, Pluske et al. (1997) highlights several changes in GIT morphology and histology PW. Emphasizing the low feed intake occurring due to change in diet as the main cause. This is supported by Nunez et al. (1996), reporting malnutrition of piglets during the development in the neonatal period altered the intestine both biochemically and morphologically, including a reduction in protein synthesis. Low feed intake during the acute phase due to weaning, may cause a deficiency of macronutrients, micronutrient and energy for the GIT, impairing health, development and recovery in the adaptive phase (Pluske 2013). In the proximal intestine, the fractional rates of protein synthesis and degradation is greatly affected by the nutrient supply, while the distal intestine derives a larger proportion of its AA needed for protein synthesis from the circulation (Stoll et al. 2000), indicating that nutrient supply is most critical in the proximal small intestine (Wijtten et al. 2011).

Weaning age, weaning stress, feed intake and diet composition have major effects on the small-intestinal barrier function post-waning (Wijtten et al. 2011), although different sections of the small intestine is affected differently (Boudry et al. 2004). A disturbed barrier function leads to increased paracellular- and transepithelial transport of macromolecules into the body. Weaning causes a loss of the paracellular barrier function, returning to PW levels after two weeks. However, in contrast to the proximal and distal jejunum, paracellular barrier function is not compromised in the ileum, emphasizing the impact of feed intake. On the other hand, transcellular barrier function for macromolecules through endocytosis improves PW (Wijtten et al. 2011).

The piglet is borne with a sterile GIT, and establishing of microbiota occurs through maternal and environmental contamination. Further, the composition of the intestinal microbiota community is heavily influenced by the substrate supply, determined by the diet (Lallès et al. 2007a). At birth the piglet is immunodeficient and is dependent on supply of immune factors in the maternal colostrum for immune protection, development and survival (Lallès et al. 2007a). Intestinal microbiota constitutes an important factor for infection protection and development of the intestinal immune system (Lallès et al. 2007b; Pluske et al. 1997; Stokes et al. 2004). The piglets' immune system of the GIT is almost fully established at seven weeks of age. Before this, they are not developed to differentiate between harmful and harmless antigens in the feed, and can get adverse immune reactions to the feed (Stokes et al. 2004). Consequently, early weaning is often accompanied by a decline in growth and increased diarrhea incidences (Lallès et al. 2007b).

By increasing the time of weaning from two to five weeks of age, Van der Peet-Schwering et al. (2007) registered greater values for red blood cells, hemoglobin, hematocrit value, mean cell volume, mean cell hemoglobin, and percentage of lymphocytes in the leukocyte, indicating healthier and more robust animals.

2.3.3 Post-weaning feeding –digestibility and performance

Post-weaning feed intake is an important factor in stimulation of digestive development in newly-weaned piglets (Makkink et al., 1994). During the first two weeks PW, the digestive tract undergo a major development (Ball & Aherne 1987). Leibbrandt et al. (1975) investigated the effect of weaning age at piglet performance, by weaning at two, three and four weeks of age. Low feed intake due to early weaning seemed to be the main reason for growth stasis PW. Although, by six weeks of age, the piglet's weight seemed independent of weaning age. Okai et al. (1976) reported increasing feed intake and weight gain by offering a feed with increased diet complexity PW. The complex starter diet reduced the PW growth decline, both when weaning at three or five weeks of age.

Feed intake depends on several mechanisms in the body, both physiological factors and sensory appraisal (McDonald et al. 2011). Introduction of creep feed in the nursing period may make the transition from milk to solid foods gentler. Although, the intake of creep feed may vary individually, consumption of creep feed stimulates early post weaning feed intake, resulting in improved performance (Bruininx et al. 2002).

The first days PW, enzyme activities in the intestinal content and pancreas are low. Low pancreatic levels immediately following weaning may be a result of reduced feed intake, indicating that increased feed intake will increase the amount of pancreatic enzymes synthesized and secreted (Owsley et al. 1986a), consequently improving digestibility and pig performance. In a comprehensive study of how age and weaning affect intestinal structure and enterocyte ability to digest and absorb nutrients, Miller et al. (1986), identify the problems in intestinal function due to weaning to be caused by changes in intestinal structure and loss off digestive enzymes, rather than changes in absorptive function.

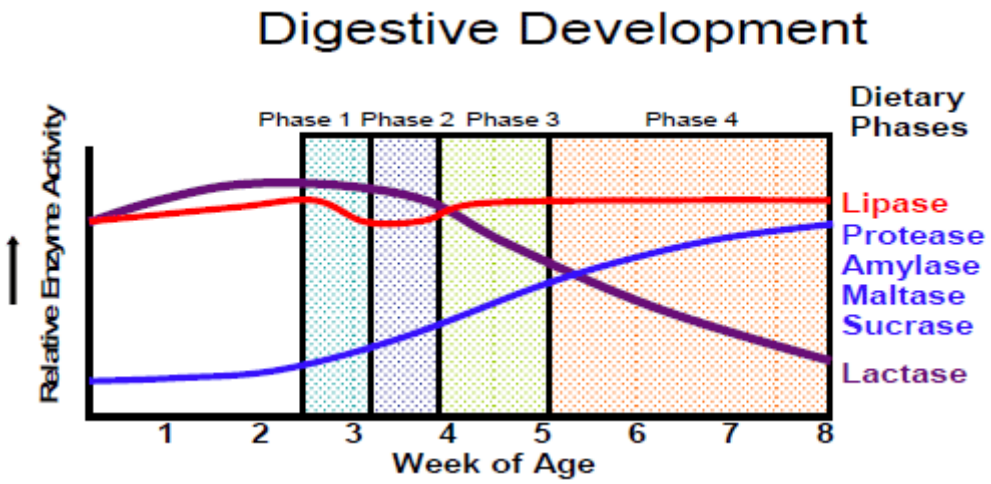


Figure 2.3 Relative enzyme activity pre- and post-weaning (DeRouchey et al. 2009).

In the nursing period, lactose (by hydrolysis to glucose and galactose) is the main energy source for intestinal epithelial cells (Spreeuwenberg et al. 2001). If not added, there are no lactose in the weaning solid-corn-based-feed. This may affect the energy supply to the intestinal mucosa before it adapts to the new diet. Weaning causes a considerable decrease in lactase activity at brush boarders in the small intestine (Hampson & Kidder 1986). Sucrase activity also decreases during weaning, but contrary to lactase, the sucrose activity recovers. Generally, there is an reduction in digestible enzymes post weaning (Pluske et al. 1997). However, production of other enzymes increases with time PW (Lindemann et al. 1986) which also might be seen as increased age (Figure 2.3). Total activity of amylase, trypsin and chymotrypsin in the pancreas and small intestine increases with age (Owsley et al. 1986a). Leading to an increased total tract digestibility, including digestibility of nitrogen, energy and dry matter, in the period after weaning (Owsley et al. 1986b).

A study in weanling piglets comparing digestibility of three different protein sources assumed to differ in digestibility, showed that a diet with dried skim milk was more readily soluble than diets with soybean meal (SBM) or corn gluten meal (Asche et al. 1989). Similarly, Owsley et al. (1986b) found that weanling piglets fed diets added dried skim milk or dried whey, increased total tract digestibility of energy and DM compared to piglets fed a basal corn-soy diet.

Raw soybeans contains antinutritional factors, where the two major are Kunitz trypsin inhibitor, inhibiting trypsin and chymotrypsin, and lectins, which are glycoproteins able to bind carbohydrate-containing molecules to epithelial cells in the intestinal mucosa (Palacios et al. 2004). Soybean protein also contains immunologically active proteins such as glycinin and β-conglycinin. Immune responses caused by the soybean protein, can result in growth setback. Improved growth performance was recorded by Palacios et al. (2004) by removing of lectin and Kunitz trypsin inhibitor, whereas inclusion of the antinutritional factor lectin, increased the loss of endogenous nitrogen, considered to be caused by damage on the intestinal mucosa. Li et al. (1990) reported a transient hypersensitivity, by decreased villus height and increased serum anti-soybean immunoglobulin in weanling piglets fed a diet containing SBM. However, the comparison in this study was milk protein, which the piglets are well adapted to digest. Dunsford et al. (1989) reported the effect was less when feeding soybean as a part of a corn-based diet, suggesting a high concentration is needed to cause detrimental effect. Whereas Owsley et al. (1986b), concluding pigs weaned at 28 days of age require at least 6 to 9 days to adjust to typical corn-SBM starter diets.

The composition and metabolic activities of the gastrointestinal microbiota is largely controlled by the diet composition (Rist et al. 2013). Inclusion of prebiotic and probiotic in weaning-feed have positive impact on reducing GIT disorders and diarrhea and may substitute and prevent use of in-feed antibiotic growth promoters (AGP) (Lalles 2008). Especially the content and composition of fermentable carbohydrates in the diet, known as prebiotics, has shown beneficial effect to intestinal health, including promote growth of beneficial Lactobacillus species and Bifidobacterium (Rist et al. 2013), and may thus increase health and performance of weanling piglets (Lalles 2008; Lallès et al. 2007b). Promoting growth of beneficial bacteria in the intestine, enhances colonization resistance against potential pathogens (Rist et al. 2013). Inclusion of probiotic such as bacteria or yeast have also provided promising results in improving intestinal health (Lallès et al. 2007b) and performance (Close 2000) in weanling piglets.

2.3.4 Post-weaning diarrhea

Post-weaning diarrhea is a condition often occurring around day 4-9 PW (Madec et al. 1998), characterized by frequent discharge of watery feces (Pluske 2012). It constitutes a major problem in the pig production, as there is a strong relationship between diarrhea, mortality and growth. Management and husbandry level such as air quality, group size and stocking procedure are important factors in preventing of PW diarrhea (Madec et al. 1998).

In a study in the Netherland, weanling pigs in herds with a long story of PW diarrhea in general had shorter villi and deeper crypts compared to pigs in specific pathogen-free herds (Nabuurs et al. 1993a). A reduction in villi height and increasing crypt depth causes decreased absorption and increased secretion due to fewer absorptive and more secretory cells. Resulting in a reduced digestion and absorption, encouraging development of an osmotic diarrhea (Pluske et al. 1997). This may be the reason Ball and Aherne (1987) and Hampson and Smith (1986) found that a high feed intake after weaning is associated with diarrhea. However, comparing a wheat-enriched diet, known to induce diarrhea more than other cereals, with a conventional diet, Boudry et al. (2004), did not find any influence of the PW diet on the changes in intestinal physiology.

Withdrawal of the sow milk in addition means removal of supply of IgA and other bioactive compounds derived from sows milk, impairing the piglets resistance to enteric diseases immediately after weaning (Heo et al. 2013). A study in herds with long history of PW diarrhea, the E.coli strain ETEC, which are typically associated with diarrhea, became predominant after weaning in 7 of 8 litters, although pre-weaning it was found only in one litter. Rotavirus was also excreted by numerous of pigs PW (Nabuurs et al. 1993b).

2.4 Yeast in diets for monogastric animals

In January 2006, the European Union (EU) banned the use of antibiotics in feed as growth promoters for livestock production (Heo et al. 2013). AGP are widely used preventing growth stasis and health problems. The high use of antibiotics in livestock production constitute a serious problem as several bacteria become antibiotic-resistant, causing a major challenge in human medicine (for review see Barton 2000). In the view of this, numerous of studies have been conducted trying to find accepted feed additives or ingredients with similar beneficial effects. Several studies have examined the possibility of using yeast as a replacement of AGP in the diet, and concluded yeast culture could be a suitable alternative to AGP (Maribo & Spring 2003; Shen et al. 2009; Van der Peet-Schwering et al. 2007; Waititu et al. 2016b). Positive effects of yeast supplementation to weanling piglets includes increased PW daily gain (Bontempo et al., 2006; Jurgens et al., 1997; Mathew et al., 1998; van der PeetSchwering et al., 2007; Shen et al., 2009; Spark et al., 2004), and advantageous parameters of the intestine health (Bontempo et al., 2006; van der PeetSchwering et al., 2007). However, the composition and functional properties of different yeast strains may vary substantially.

2.4.1 Yeast as a protein source

Most studies have revealed yeast as a probiotic and feed additive with beneficial effects. Although, yeast has a high nutritional value and may thus be used as a protein feed ingredients, as described in chapter *2.1.4 Yeast in feed*. The content of nutrients and the digestibility of yeast differ with production technology, substrate and strain (Czech et al. 2016). Kats et al. (1994) reported differences in piglet performance due to the processing method. Spray-drying of blood meal resulted in an improved protein quality relative to flash-drying. Similar results was found in a study by Spark et al. (2005), with three different yeast strains in piglet feed.

Maribo and Spring (2003), studied yeast extract as a protein source for weanling piglets, included at a 2.5 % level in the diet, replacing fishmeal and whey. Piglets fed the diet with yeast had improved weight gain and feed intake, and a reduced mortality. Moreover, studying yeast products from the ethanol industry, Kim et al. (2014), reported greater values for metabolic energy in yeast than in corn, fish and SBM, concluding the yeast successfully could be included as a protein source in diets for growing pigs. In addition, they found greater standardized total tract digestibility of phosphor in the yeast compared to SBM. Recent studies have also shown yeast to be a suitable protein source for carnivore fish (Øverland et al. 2013) and shrimps (Zhao et al. 2015).

2.4.2 Performance and digestibility

S. cerevisiae, also called "Baker's yeast" is one of the most widely commercialized types of yeast (Zhang et al. 2005). It is a yeast commonly used and tested as a growth promotor, function as probiotics. Other yeast strains, such as *Yarrowia lipolytica* (Czech et al. 2016), *Kluyveromyces marxianus* and *Candida utilis* is also proven to be suitable as feed ingredients (Øverland et al. 2013). All these yeasts have obtained the generally-regarded-as-safe (GRAS) status assigned by the US Food and Drug Administration (Øverland & Skrede 2016) and are approved as feed ingredients by the European Food Safety Authority.

Feeding a baker's yeast derived protein, Hu et al. (2014) reported an increased daily weight gain and lower final feed conversion ratio (FCR) in weanling piglets. Likewise, Shen et al. (2009) reported dietary supplementation of 5 g/kg of yeast culture improved average daily gain (ADG) compared to a control group, and Van der Peet-Schwering et al. (2007) found an improved FCR for piglets fed yeast supplemented diets. Additionally, in a study by Le Bon et al. (2010), inclusion of yeast as a probiotic in piglet feed for 9-weeks PW improved FCR compared to a control diet. Dietary yeast (*S. cerevisae*) has also shown to increase growth performance in broilers (Zhang et al. 2005). By contrast, other studies have revealed no effect of dietary yeast on PW performance (Kornegay et al. 1995; Yang et al. 2016)

Yeasts can differ greatly in digestibility of organic matter, but in general have a good digestibility of crude protein (Schulz & Oslage 1976). However, some animals may have difficulties digesting intact yeast cells. In some fish species, it has been reported a lower digestibility of intact yeast, whereas by removal of the cell wall the yeast extract have provided a greater digestibility (Øverland & Skrede 2016). However, the cell wall fraction is rich in bioactive and immunostimulating compounds with health promoting effects, and the whole yeast may therefore be the most attractive feed ingredient (Øverland & Skrede 2016).

Other types of SCP may also be suitable as feed ingredients and improve pig performance. Studies have shown positive effect on growth performance by using BPM in diets for weanling piglets (Øverland et al., 2001). A digestibility study with BPM in diets for mink, chicken, pigs and salmon by Skrede et al. (1998), showed a general similarity among the species for digestibility in the small intestine and utilization of the AA of BPM. In pigs, total tract digestibility showed a higher digestibility for arginine, lysine and glutamic acid, and a lower digestibility for cysteine, phenylalanine, tyrosine and serine, than total N digestibility.

Animal proteins, including yeast and bacteria, in general are highly digestible and have a well-balanced amino acid profile (Maribo & Spring 2003). In a study by Shen et al. (2009), supplementing of yeast culture in diets to weanling piglets, improved total tract digestibility of DM, CP and gross energy (GE). Similarly, by inclusion of increasing amount of whey yeast (*Kluyveromuces fragilis*), Spark et al. (2005) reported an increased N-digestibility and N-retention. Moreover, inclusion of increasing amount of yeast in broilers diet, increased digestibility of Ca and P (Gao et al. 2008). In dogs, supplementation of yeast in the diet increased ileal digestibility of DM, OM, CP and GE (Middelbos et al. 2007). However, no effect was observed on total tract digestibility. Other studies, by contrast, revealed no effect (Kornegay et al. 1995; Veum & Bowman 1973) or a decreased digestibility (Van Heugten et al. 2003) of nutrients in diets with yeast supplementation.

2.4.3 Influence of yeast on the intestinal health

Pigs fed a diet with live yeast supplementation PW had greater villus height and crypt depth (Bontempo et al. 2006; Van der Peet-Schwering et al. 2007). They also had a thicker intestinal mucus layer, greater proliferating epithelial cell counts and a higher number of mucosal macrophages (Bontempo et al. 2006). These findings indicate an early restoration of the intestinal changes due to weaning (as described previous in chapter *2.3.2 Development of the digestive tract in the weanling piglet*), and a possibly improved local infection resistance in piglet fed yeast supplementation. Shen et al. (2009) reported an increase in villus height and villus:crypt ratio, but only in the jejunum. By contrast, in a study with yeast culture and modified yeast culture in diets for weanling pigs, Van der Peet-Schwering et al. (2007) registered no effect of the dietary treatment on blood cell composition, villous length or crypt depth. Moreover, Rigueira et al. (2013), also reported no differences in villous height or crypt depth in piglets receiving a pre- and post-weaning diet supplemented with yeast or plasma, compared to a control diet. However, studying electron-micrographs they did see a more flattened villi in the pigs fed the control diets, indicating a poorer utilization of dietary nutrients. Conflicting results may be due to differences in the methods such as weaning age, feeding regime, diet composition, and quantity and strain of yeast included in the diet. In addition, differences in sanitary conditions of the animals may affect the results.

The cell wall of the yeast is comprised of polysaccharides (Kogan & Kocher 2007) consisting mostly of β(1,3) and β(1,6) glucan, mannoproteins and chitin (Lipke & Ovalle 1998). The structure of the yeast cell wall are shown in Figure 2.4. The yeast β(1,3) glucan is known as a "biological response modifier", which increases the host resistance to disease by stimulating the immune system in a non-toxic way to the cells of the host organism (Moran 2004). Davis et al. (2004), fed weanling piglets a diet with phosphorylated mannans derived from the yeast cell wall of *S. cerevisiae*, reporting beneficial effects on performance and immune function. In broilers, supplement of yeast culture has been shown to affect immune functions by increasing antibody titers (to Newcastle disease virus), serum lysozyme activity, IgM, and secretary IgA concentrations in the duodenum (Gao et al. 2008). It is suggested yeast is capable of inhibiting toxic effects by mycotoxin adsorption properties (Kogan & Kocher 2007), presumably dependent of the glucan concentration in the cell wall (Moran 2004).

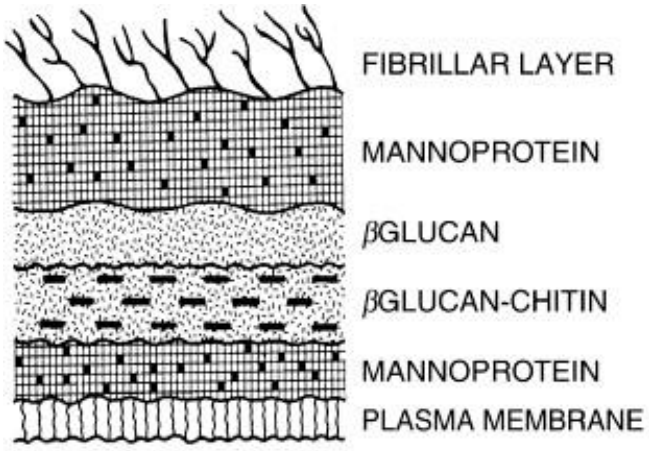


Figure 2.4 Structure of the yeast cell wall showing the major polysaccharide components (Kogan & Kocher 2007).

A review by Sauer et al. (2011), is discussing the role of dietary nucleotides in monogastric animals. Nucleotides constitutes monomers in DNA and RNA, energy transfer molecules such as ATP, and physiological mediators such as AMP. Hence, nucleotides are especially important in tissue with a high turnover rate, such as the intestinal mucosa and lymphoid tissue. The potential sources of nucleotides is from de novo synthesis, recycling through salvage pathways, and the diet (Boza 1998). In the gastrointestinal tract, dietary nucleotides are metabolized and will presumably not entry the systemic circulation. However, by low feed intake due to weaning or in periods with rapid growth and maturation, endogenous supply of nucleotides

may be insufficient for normal function. Thus, a exogenous source of nucleotides from the diet may optimize the function of this rapidly dividing tissues (Carver 1994). In the weanling period of rats, supplementation of nucleosides seemed to enhance gut growth and maturation of the intestine (Uauy et al. 1990). Dietary nucleotides may also contribute to maintenance of the immune response, by stimulating the humoral and cellular immune system of the GIT (Carver 1999). However, Waititu et al. (2016a), found that increasing levels of nucleotide-rich yeast extract (1000 ppm and 2000 ppm), had no effect on ADG and gain to feed ratio, but resulted in similar growth performance as in diets including antimicrobial growth promoters (AGP).

In rats, it is shown that a lack of dietary nucleotides negatively influences protein synthesis in the liver and small intestine (Sanchez-Pozo & Gil 2002). Consequently, this may impede maturation (Ortega et al. 1995). Similarly, nucleotides may be important nutrient for intestinal repair, such as after chronic diarrhea, and may be considered as a "semi-essential" nutrient for the intestine (Bueno et al. 1994) In a study by Martinez-Puig et al. (2007), supplementation of nucleotides from yeast in the diet for weanling piglets reduced the incidence of PW diarrhea.

Waititu et al. (2016b) challenged weanling piglets with E.coli lipopolysaccharide, reporting supplementation of yeast extract in the diet seemed health promoting for the weanling piglets, including beneficial immunoregulatory responses. Le Bon et al. (2010) found reduced levels of E.coli in piglets fed yeast as a probiotic for 9 weeks PW. Whereas, by dietary supplementation of yeast culture, Shen et al. (2009) reported a decrease in E.coli in the cecum. However, no effect of yeast supplementation was found in the colon, or in terms of lactobacilli counts. Lactobacilli is known through their favorable effects on the microflora of the intestine (Bernardeau et al. 2006). By contrast, in a study by (Hu et al. 2014), weanling piglets fed a diet with yeast-derived protein had increased amount of lactobacilli and total bacteria in the colon. A greater count of lactobacilli in the intestine in piglets fed yeast was also found by White et al. (2002). Other studies, on the other hand, reported no effect of yeast supplementation on intestinal microflora (Mathew et al. 1998; Van der Peet-Schwering et al. 2007).

2.4.4 Candida utilis

C. utilis is a strictly aerobic yeast, also known as Torula yeast, and have been used as nutritional supplement in animal feeds for more than 70 years. *C. utilis* is also fully accepted for human consumption (Bekatorou et al. 2006). Recent studies has shown promising result for *C. utilis* as a protein source for Atlantic salmon (Øverland et al. 2013). *C. utili*is also shown to counteract enteropathy in Atlantic salmon (Grammes et al. 2013), corresponding to Miadoková et al. (2006), concluding that glucomannans from *C. utilis* have a broad range of biomodulatory properties.

C. utilis can be grown on different substrates, including lignocellulosic biomass (Parajó et al. 1995; Øverland & Skrede 2016), molasses, waste and brewing products (Bekatorou et al. 2006). In a study by El-Deek et al. (2009) *C. utilis* was used for converting dried poultry manure to a protein source in the diet with increased true protein content for chickens, concluding inclusion up to 9 % in broiler diets could be done without adverse effects.

2.5 Summary of literature & aim of thesis

As reviewed, weaning causes several changes in the digestive tract that may affect digestion and absorption of the post-weaning feed. In addition, the digestive tract undergo an extensive maturation occurring at the same time as the weaning. Consequently, post-weaning diarrhea is commonly occurring in piglets. Yeast may help the piglets to better cope with the transition of feed, by improving the intestinal health.

The aim of this thesis is to evaluate the effect of increasing dietary level of yeast biomass on nutritional value (nutrient digestibility and growth performance) and general health responses (e.g., fecal scores, general blood parameters) in early weaned piglets. In view of the previous literature review, there are reasons to assume that (the *Candida utilis*) yeast may be a suitable protein source with beneficial effects on health and thereby performance of the weanling piglets.

Based on the literature review of previous studies, the following hypothesis is predicted:

H0: *Candida utilis* yeast may replace high quality protein sources in feed for weanling piglets.

This is a result of the following sub-hypotheses examined in this thesis:

H1: Inclusion of dietary yeast will result in improved performance of the piglets.

H2: Inclusion of dietary yeast will improve digestibility of the feed and metabolic utilization of nutrients (will be examined further in a follow up experiment with the same dietary treatments).

H3: Inclusion of yeast in the feed may improve the intestinal health, and reduce the incidence of post-weaning diarrhea.

3.0 Materials and Methods

The experiment was carried out at Ås Farm, the Animal Production Experimental Centre (SHF), at the Norwegian University of Life Sciences (NMBU), Ås, Norway, between 21th of February to 21th of March 2017. The experiment is a part of a comprehensive study; the *Effect of Candida utilis as a protein source with immunomodulatory functions in diets for piglets*, conducted by Foods of Norway, a Centre for Research-based Innovation at the NMBU, Norway. The experiment was designed as a dose-response experiment with increasing amounts of yeast in the diets, and was approved by the Norwegian Food Safety Authority.

3.1 Production of yeast

Wood chips from Norwegian spruce trees was used in a biorefinery process (BALI process (Sjöde et al. 2011; Sjöde et al. 2013; Sjöde et al. 2015)) at the Borregaard pilot plant in Sarpsborg, Norway, to produce a solution containing primarily monosaccharides (C5 and C6 sugars). The BALI-sugars was mixed 1:1 with sugars from beet molasses and used as the principal carbon source to promote yeast growth. The yeast (*C. utilis*) was grown in a 42.000-Liter fermentor at the Lallemand plant in Salutaguse, Estonia. After fermentation, the yeast cells were washed, centrifuged and heat-inactivated before drum drying.

3.2 Feed production

The feed was manufactured by the Center for Feed Technology (FôrTek), NMBU, Ås, Norway. A control diet (0 % yeast) and three diets containing increasing amounts of yeast (corresponding to 10, 20 and 40 % of total CP in the diet) were used in this experiment. In addition, the same four diets containing an inert marker (0.01 % Yttrium(III)oxide: Y_2O_3) were produced for determination of digestibility. The formulation of diets was done in collaboration with Felleskjøpet Fôrutvikling, using their optimization least-cost program. All diets were formulated to meet or exceed the requirements for indispensable AA and energy for this age pig (NRC 2012). All diets were grain based with protein sources being soybean meal (SBM), fishmeal, potato protein, rapeseed cake and yeast (not in control). Chemical composition of main ingredients are shown in Table 3.1. Composition of the diets are shown in Table 3.2.

Feed ingredients were grinded at 3 mm sold in a hammer mill. Steam was used to warm the feed up to 81°C to avoid salmonella contamination, according to Norwegian law. Pellet diameter was 3 mm. Producing the diets with the two highest levels of yeast demanded higher power consumption and motor load p.press than the other diets (Appendix I). In addition, the pellet temperature differed between the diets, from 82.7 °C in Yeast 40 to 93.1°C in Yeast 20.

FôrTek performed pellet quality analyses of the feed; testing dry matter and durability of the pellet. Pellet durability index was determined using a Holmen NHP200 Pellet Durability Tester. Increasing level of yeast gave a higher pellet durability index, while the moisture content decreased. Results are presented in Appendix I.

Table 3.1 Chemical composition of main feed ingredients.

	Chemical composition					
Item	DM g/kg	Ash g/kg	ADF g/kg	CP g/kg	CF g/kg	Energy MJ/kg
Wheat	861.6	13.7	34.1	98.4	12.2	15.9
Barley	863.2	18.4	59.6	79.4	14.2	15.9
Oats	849.7	24.0	122.9	88.1	44.9	16.7
Soybean meal[1]	881.3	56.3	89.0	457.9	9.6	17.5
Fishmeal[2]	916.5	145.1	5.1	683.7	73.2	19.4
Rapeseed meal[3]	888.7	59.0	161.1	349.9	88.1	19.1
Yeast meal (*Candida utilis*)	970.1	77.8		470.0	16.0	19.9

[1] Non-GMO soybean meal, Denofa AS, Fredrikstad, Norway.

[2] Norsildmel AS, Egersund, Norway.

[3] Expeller-pressed rapeseed cake from Mestilla, Lithuania (supplied by Felleskjøpet Rogaland & Agder, Stavanger, Norway).

Table 3.2 Dietary composition and calculated protein content of experimental diets.

	Dietary treatments			
Ingredient, g/kg as fed basis	Control	Yeast 10	Yeast 20	Yeast 40
Wheat[1]	622.8	615.4	607.4	591.7
Barley[1]	100.0	100.0	100.0	100.0
Oats[1]	50.0	50.0	50.0	50.0
Soybean meal[1]	80.0	65.3	50.2	19.2
Potato protein conc.[1]	37.8	30.0	23.3	9.1
Fish meal[1]	20.0	16.2	12.5	4.8
Rapeseed meal[1]	20.0	16.0	12.2	4.9
Yeast - *Candida Utilis*[1]	—	36.2	72.6	146.0
Rapeseed oil	21.5	22.4	23.2	24.9
Monocalcium phosphate	13.1	13.7	14.3	15.5
Limestone	9.2	9.2	9.3	9.4
Sodium chloride	6.3	6.0	5.6	4.9
Selenium premix	0.7	0.8	0.8	0.9
Iron(II) fumarate	0.4	0.4	0.4	0.4
Micro-mineral premix[2]	2.0	2.0	2.0	2.0
Vitamins[3]	2.1	2.1	2.1	2.1
L-Lysine	6.3	6.3	6.1	5.8
L-Methionine	2.1	2.3	2.5	3.0
L-Threonine	2.8	2.8	2.6	2.4
L-Valine	1.0	1.0	1.0	1.0
L-Tryptophan	0.9	0.9	0.9	1.0
Yttrium (III) oxide	1.0	1.0	1.0	1.0
Calculated protein content (%)	17.0	17.0	17.0	17.0
Ratio CP from yeast (% of total CP)	0.00	10.0	20.1	40.3

[1] Chemical composition of main protein ingredients; please see Table 3.1.

[2] "Mikro svin"; provided per kilogram of diet: 475 mg Ca; 3.4 mg Mg; 13.2 mg S; 120 mg Fe; 60 mg Mn; 120 mg Zn; 26 mg Cu; 0.6 mg I.

[3] Provided per kilogram of diet: 0.8 g Vitamin A; 0.3 g Vitamin E; 0.8 g Vitamin ADKB mix; 0.3 g Vitamin C (Stay C 35%).

3.3 Animals

A total of 48 crossbred [(Norwegian Landrace×Yorkshire)×(Duroc) and (Norwegian Landrace)×(Duroc)] weanling piglets from 12 litters were selected for this trial. Weaning was conducted at 4 weeks of age (27-30 days of age). All piglets in the litters were weighted, and four piglets from each litter, with an average initial body weight (BW) of 11.06 kg (ranging from 9.8 to 13.5 kg) were selected. Piglets that had received medical treatment were excluded. Piglets were allotted to the dietary treatments based on litter, initial weight and sex. There were a total of 23 gilts and 25 boars. Piglets were randomly distributed into 12 pens; 3 pens for each dietary treatment, and 4 pigs in each pen. However, initial body weight was taken into consideration to minimize the differences between dietary treatments, as well as the gender balance in the pens. Each pen received the same diet, and dietary treatments were distributed throughout the room, in case of disease contamination between the pens. Live bodyweight of all experimental pigs were recorded weekly.

3.4 Pens

Piglets were housed in an environmentally controlled room. The pens had partially slatted concrete floors and six feeding boxes for individual feeding (Figure 3.1). Two of the boxes remained closed. The piglets had ad libitum access to water from drinking nipples, localized by the manure area with slatted floor, and between two feeding stalls. Since straw bedding may influence the digestibility, the pens were installed with rubber mats, and toys were offered in each pen. Heating lamps were installed over the rubber matt to provide a comfortable resting area. Temperature in the room was logged every day, and was on average 19.1°C.

Figure 3.1 Photo of pen prior to the experimental period.
Photo: Ingrid Marie Håkenåsen.

3.5 Feeding routines

In the nursery period, piglets had access to creep feed by using the same diets as the sow. In the experiment, piglets were fed the experimental diets individually by using feed drop boxes. The piglets received their first meal with experimental diet the day after weighing and grouping. Piglets were trained, using positive reinforcement, to find their regular eating-place to allow for individual feed intake. Piglets had access to clean drinking water from drinking nipples in the pen, and during feeding from approximately day 10.

The experimental diets were fed three times per day, at 08:00, 11:00 and 14:00 h in the two first week, but changed to two times per day at 08:00 and 14:00 h, the last two weeks of the experimental period. Piglets were fed semi-ad libitum. Any leftovers after the meal were collected. Collected leftovers were weighed and registered once a week. The piglets received feed corresponding to 5% of their BW per day, divided on 2 or 3 equal meals. The feeding intensity was adjusted once a week according to BW.

3.6 Fecal scoring

Every day during the experiment, fecal consistency in the pens was assessed by using the scoring system developed by Pedersen and Toft (2011). Individual fecal samples in the pen were assessed, but the scoring was given as an average of each pen, based on the following four consistency categories: score 1 = firm and shaped, score 2 = soft and shaped, score 3 = loose, and score 4 = watery (Figure 3.2). Samples with score 1 and 2 were considered as normal. Samples with score 3 or 4 were considered diarrheic.

People responsible for daily scoring was familiarized with the classification scale. The classification scale was available for comparison during the registration. It was attempted that the same person did the assessment and scoring during the completely experimental period, but it was not feasible. According to Pedersen and Toft (2011), the classification scale does not prevent variation between observers. However, it could improve inter-observer agreement. Thus, there may also be a variation in intra-observer agreement

Fecal samples were collected at day 7, 14 and 21, for determination of dry matter content. From each pen, a representative sample was collected and stored in aluminum boxes, marked with pen and date. Boxes with samples were immediately stored in the freezer at -20°C. Later, samples were weighed and oven-dried at 103°C for determination of dry matter content.

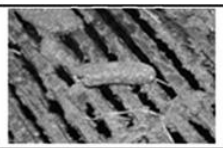
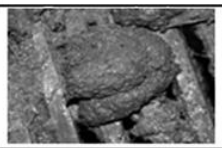
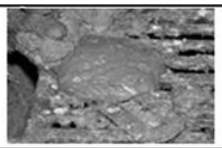
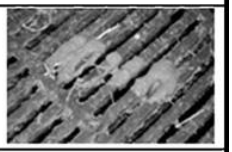
Score	1 Firm and shaped	2 Soft and shaped	3 Loose	4 Watery
Picture				
Texture	Firm. Varies in hardness.	Varies in softness. Like peanut butter	Mush. Often shining surface	Varies form gruel to water.
Shape	Sausage	Varies form sausage shape to small piles	Tends to level with surface. Does not flow through or flows slowly through slatted floors.	Levels with surface. Flows through slatted floors.
In container	Preserves original shape.	Does not flow when container is rotated. Preserves original shape.	Inert when container is rotated. Merges and covers bottom of container in most cases.	Flows easy when container is rotated. Merges and covers bottom of container.

Figure 3.2 Fecal classification scale with 4 categories, descriptive text and pictures (Pedersen & Toft 2011).

3.7 Sampling of blood

At day 7, blood from the jugular vein was collected from half (24) of the piglets. Sampling was done after morning feeding. A trained person performed the sampling. Piglets was selected on the basis of feed intake and those who visually seemed strong and in good health. Hence, those who seemed best suited to handle the stressful situation with blood sampling. Termination day, blood from the jugular vein was collected from the same pigs, but only the ones who had received the control and Yeast 40 diets. Whole blood for hematology analyses was sampled in EDTA tubes, carefully mixed to avoid coagulation, and then shipped to the Central laboratory of NMBU, Oslo, Norway, for analyses.

3.8 Sampling of feces for determination of total tract digestibility

Piglets received feed labeled with Y_2O_3 digestibility marker from day 18. Feces were collected over a period of 5 days (day 21-25; March 14th to 18th). Fecal samples from each individual pig was collected after the morning meal (08:00 h), when each piglet was separated in the feeding boxes, to avoid mixing of samples. If it was not possible to get a sample from the pig in the morning, a second attempt was done during afternoon feeding (14:00 h). Samples were collected in pre-labelled and tared aluminum boxes with cover, marked with pig ID, pen number and date. Boxes with samples were immediately stored in the freezer at -20°C. All samples from the same pig were stored in the same box. At the end of the collection period, the box with all the samples were freeze-dried and grinded at 1 mm using a hammer mill and at 0.5 mm using a pin mill, for further chemical analyzes of nutrients.

3.9 Sampling of ileal digesta for determination of ileal digestibility

At the end of the experiment, all pigs were sacrificed. Ileal content was squeezed out of the last two meters of the ileum, collected in a pre-marked an tared aluminum box, and immediately frozen at -20°C. If the sampled ileum had very little content, an additional meter of the ileum was collected as a reserve. Ileal content were then freeze dried, homogenized by using a coffee grinder, and chemically analyzed.

3.10 Chemical analysis

The LabTek group at the Department of Animal an Aquacultural Science, NMBU, Ås, Norway, conducted chemical analysis of feed, ileal and fecal samples. All ingredients and diets were grinded at 1 mm and 0.5 mm for chemical analysis of main nutrient content. The diets were analyzed in triplicates (except AA and tryptophan) for: DM, ash, starch, CP, CF, NDF, energy content, AA and tryptophan. Results in Table 4.1. Fecal samples were analyzed in duplicates for: DM, ash, starch, CP, CF, NDF and energy content. Ileal samples were analyzed in duplicates (except AA and tryptophan) for: DM, ash, starch, CP, AA and tryptophan.

- The diets, ileal, and fecal samples were analyzed for dry matter by drying to constant weight at 104 °C. Ash were determined by complete combustion at 550°C in at least 4h.
- Crude protein (CP) was analyzed using Kjeldahl nitrogen x 6.25 according to Commission regulation (EC) No 152/2009, using a 2400/2460 Kjeltec^{TM} Auto Sampler and the Kjeltec 1015 Digester Tecator (FOSS Analytical, Hilleroed, Denmark).

- Neutral detergent fiber (NDF) was analyzed according to Mertens (2002) using the Ankom200 Fiber Analyzer (ANKOM Technology, Macedon, New York, USA).
- Gross energy (GE) content was determined by using a PARR 1281 Adiabatic Bomb Calorimeter (Parr Instruments, Moline, IL, USA) according to ISO 9831 (1998).
- Crude fat was determined using Accelerated Solvent Extraction (ASE350, Dionex Corporation, Sunnyvale, CA, USA), according to the manufacturer's instructions (Dionex 2010). Feed samples were extracted with 70 % petroleum ether and 30 % acetone at 125°C, whereas ileal and fecal samples were extracted with 80 % petroleum and 20 % acetone at 125°C.
- Starch was determined using an enzymatic-colorimetric method according to McCleary et al. (1994). In brief, starch was degraded with α-amylase and amyl glucosidase-enzymes to glucose. Glucose concentration was then determined using a spectrophotometer (MaxMat PL II Multianalyzer, France).
- Amino acids (except tryptophan) in diets and ileal samples were analyzed according to Commission regulation (EC) No 152/2009 on a Biochrom 30 Amino Acid Analyzer (Biochrom Ltd., Cambridge, UK). Tryptophan was analyzed according to Commission regulation (EC) No 152/2009 on a Dionex UltiMate 3000 HPLC system (Dionex Softron GmbH, Germering, Germany) with a Shimadzu RF-535 fluorescence detector (Shimadzu Corporation, Kyoto, Japan).

Analysis for marker in all feed, ileal and fecal samples were conducted at the Department of Environmental Sciences, NMBU. In brief, Yttrium (Y-89) concentrations in diets and dried ileal and fecal digesta was determined by inductively coupled plasma mass spectroscopy using an Agilent 8800 Triple Quadrupole ICP-MS/MS (Agilent Technologies Inc., Santa Clara, USA) in oxygen reaction mode. The samples were completely digested in concentrated nitric acid (HNO_3) in an UltraCLAVE III (Milestone, Sorisole, Italy) at 260 °C for 15min, and diluted with deionized water before analysis.

Standard hematology analysis of blood samples was conducted by the Central laboratory of NMBU, Oslo, Norway. Reference area for pigs are stated at the website of the Central laboratory (Sentrallaboratoriet 2010):

http://www.sentrallaboratoriet.no/informasjon-om-referanseomradene/referanseomrader-gris/

3.11 Calculations

3.11.1 Performance

$$\text{Average daily gain (g)} = \frac{BW\ day\ y\ (kg) - BW\ day\ x\ (kg)}{number\ of\ days} \times 1000$$

Average daily feed intake (g) =

$$\frac{Feed\ given\ from\ day\ x\ to\ y\ (kg) - residues\ from\ day\ x\ to\ y\ (kg)}{number\ of\ days} \times 1000$$

$$\text{Feed conversion ratio (FCR)} = \frac{ADFI\ (g/dag)}{ADF\ (g/dag)}$$

3.11.2 Liver index

$$\text{Liver index} = \frac{Liver\ weight\ (kg)}{BW\ (kg)} \times 100$$

3.11.3 Digestibility

Apparent digestibility coefficients were calculated using the formula of Maynard and Loosli (1969).

Apparent total tract digestibility coefficient (%) =

$$100 - \left(100 \times \left(\frac{nutrient\ in\ feces\ (g/kg\ DM)}{nutrient\ in\ diet\ (g/kg\ DM)} \times \frac{marker\ in\ diet\ (g/kg\ DM)}{marker\ in\ feces\ (g/kg\ DM)}\right)\right)$$

Apparent ileal digestibility coefficient (%) =

$$100 - \left(100 \times \left(\frac{nutrient\ in\ ileal\ content\ (g/kg\ DM)}{nutrient\ in\ diet\ (g/kg\ DM)} \times \frac{marker\ in\ diet\ (g/kg\ DM)}{marker\ in\ ileal\ content\ (g/kg\ DM)}\right)\right)$$

3.12 Statistical analyses

Statistical analyses were performed using the GLM procedure of SAS® 9.4 software. Each pig was the experimental unit. Treatment, litter and sex were included in the statistical model as explanatory effects.

Statistical model:

$$Y_{ijkm} = \mu + \alpha_i + \beta_j + \tau_k + \varepsilon_{ijkm}$$

Where Y_{ijkm} is the dependent variable (pig), μ = overall mean, α_i = treatments effects (i= 1, 2, 3, 4), β_j = litter effect (1,2,..12), τ_k = effect of sex (1,2) and ε_{ijkm} = residual error.

Data for performance, digestibility and liver index were analyzed using the model above. Sex were found to have no effect in explaining performance data and were excluded in the analysis. Litter were found to have no explanatory effect on liver index and was excluded in the analysis. For hematology results, a univariate analysis was performed, only including dietary treatment.

In statistical analyses of fecal DM and fecal scoring, pen constituted the experimental unit (Y_{ij}), and diet the explanatory effect, using the following model:

$$Y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$$

Where Y_{ij} is the dependent variable (pen result), μ = overall mean, α_i = treatments effects (i= 1, 2, 3, 4), ε_{ij} = residual error.

Effects are considered statistically significant for $P \leq 0.05$ and tendencies are defined as levels between 0.05 and 0.10.

Results are presented as the least square mean (LSMEANS) for each treatment, and variance in the data are expressed as standard error of the mean (SEM). Differences between means were tested using the GLM contrast statement (orthogonal contrasts); linear (-3 -1 1 3), quadratic (1 -1 -1 1) and control vs yeast (-3 1 1 1).

Statistically significances and tendencies in contrasts are shown in tables as:

* = tendency linear regression	** = significant linear regression
+ = tendency quadratic regression	++ = significant quadratic regression
# = tendency control vs yeast	## = significant control vs yeast

In the statistical analysis of the performance and AID data extreme outliers, defined as >3*IQR (interquartile range) from the average, were excluded from the data set. Performance results including all data are shown in appendix II.

One pig was excluded from the ATTD dataset due to an unlikely high analyzed yttrium value in the feces (>3*IQR from mean value) affecting all the ATTD results for this pig.

4.0 Results

4.1 Feed

Table 4.1 Analyzed chemical content of diets.

	Dietary treatments			
Nutrients, g/kg	Control	Yeast 10	Yeast 20	Yeast 40
DM	891.21	894.77	897.43	896.43
Crude protein	173.00	174.56	167.38	174.58
NDF	98.61	90.98	81.36	72.89
Starch	494.77	498.79	506.29	489.61
Ash	48.10	50.73	48.96	53.43
Crude fat	36.45	35.10	37.60	44.33
Energy, MJ/kg	16.77	16.89	16.91	16.96
Indispensable AA, g/kg				
Arginine	9.33	9.06	8.84	8.65
Histidine	3.72	3.59	3.51	3.35
Isoleucine	7.09	6.76	6.84	6.57
Leucine	12.47	12.06	11.72	11.22
Lysine	13.13	13.01	12.78	12.33
Methionine	4.41	4.46	4.49	4.74
Phenylalanine	7.90	7.64	7.37	7.00
Threonine	9.50	9.62	9.31	9.46
Tryptophan	2.83	2.86	2.86	2.81
Valine	9.48	9.28	9.20	9.08
Dispensable AA, g/kg				
Alanine	7.21	7.30	7.33	7.80
Aspartic acid	14.36	13.81	13.19	12.60
Cysteine	2.56	2.46	2.37	2.16
Glutamic acid	34.96	34.78	34.66	34.28
Glycine	7.63	7.38	7.21	6.96
Proline	11.88	11.66	11.57	10.87
Serine	8.53	8.61	8.30	8.40
Tyrosine	3.05	3.29	3.09	3.07

Analyzed content of nutrients is quite similar between the diets (Table 4.1), which corresponds to the intention of the diets formulations. The largest variation is in the content of NDF, decreasing with increasing levels of yeast, consequently because the other protein sources contains more NDF than the yeast. Yeast 20 contain some lower CP content than the other diets. Energy content in the different dietary treatments are approximately the same. For some of the AA the content is decreasing by increasing levels of dietary yeast.

4.2 Performance

The feed intake was very low during the first days PW and some pigs did not eat at all. This was probably due to the stress related to change in diet and environment. In addition, being placed in the feeding stables was very stressful for the pigs in the beginning, but after few days, they calmed down and started to eat well. In about one to two weeks, all the pigs had learned to find their regular feeding stable.

As expected, litter affected ADG (P=0.065) and ADFI (P<0.05), in the four-week experimental period (except first week).

Table 4.2 Effect of increasing levels of yeast in diets on performance of weanling piglets.

		Dietary treatments[2]						
Week		Control	Yeast 10	Yeast 20	Yeast 40	SEM	P-value	R[1]
0-1	ADG g/d	108	104	144	176	26.9	0.193	**
	ADFI g/d	201[ab]	182[a]	218[b]	206[ab]	11.2	0.133	
	FCR	1.61	2.50	1.07	1.28	0.550	0.276	
0-2	ADG g/d	181	175	208	195	12.8	0.230	
	ADFI g/d	275[ab]	263[a]	294[b]	274[ab]	11.2	0.241	
	FCR	1.59	1.53	1.49	1.41	0.071	0.369	*
0-3	ADG g/d	269	265	295	285	11.4	0.223	
	ADFI g/d	368[a]	361[a]	403[b]	377[ab]	12.2	0.095	
	FCR	1.38	1.37	1.38	1.33	0.029	0.580	
0-4	ADG g/d	334	328	329	350	12.5	0.555	
	ADFI g/d	457[a]	457[a]	496[b]	465[ab]	13.1	0.133	
	FCR	1.38[a]	1.41[a]	1.52[b]	1.34[a]	0.033	0.004	++

[1]* = tendency linear regression ** = significant linear regression ++= significant quadratic regression
a, b – values in the same rows with different letters differ significantly at P≤0.05.
[2]Extreme outliers (>3*IQR from average) are excluded from the data.

Piglets receiving the diet with 10 % yeast (Yeast 10), had the numerically, but not significant, lowest ADG and ADFI all weeks throughout the entire experimental period (Table 4.2). Whereas ADFI in general was highest for the diet with 20% yeast (Yeast 20). The first week PW, a linear increase in ADG (P<0.05) with increasing inclusion of dietary yeast in the diet was observed (Figure 4.1).

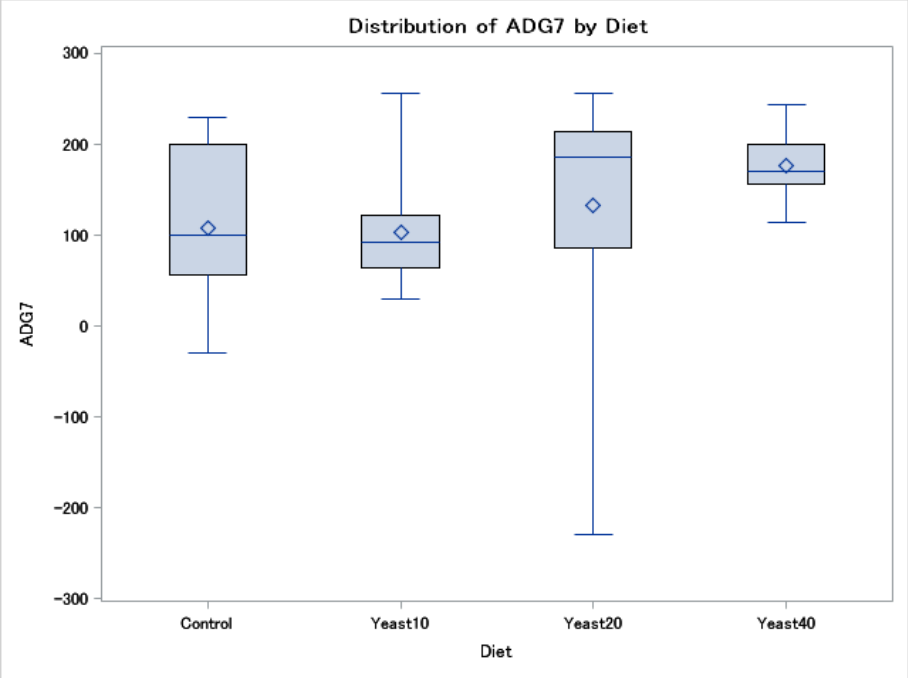


Figure 4.1 Distribution of ADG by dietary treatment, the first week PW.

There was a tendency for a linear correlation in FCR the two first weeks (P=0.081); FCR decreased by increasing level of dietary yeast. For the overall period, there was a quadratic correlation between FCR and level of dietary yeast (P<0.05; Figure 4.2). Yeast 20 had significantly higher FCR compared to the other diets, and Yeast 40 the numerically lowest FCR (Table 4.2).

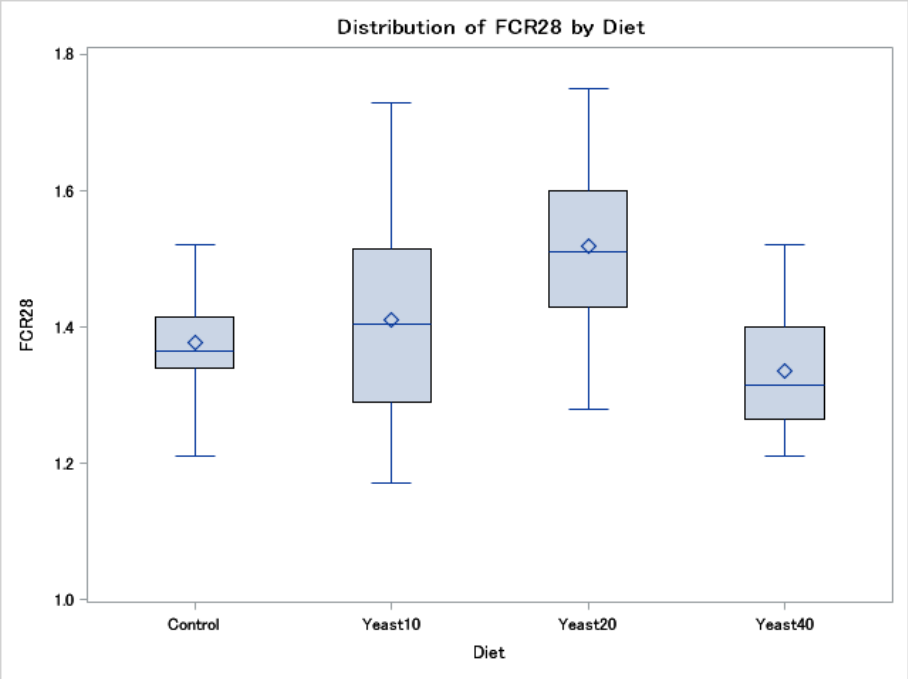


Figure 4.2 Distribution of the FCR for the overall period.

4.3 Digestibility

4.3.1 Apparent ileal digestibility coefficients (%).

Table 4.3 Effect of increasing levels of yeast in diets on AID of main nutrients in weanling piglets.

	Dietary treatments[2]						
Nutrients	Control	Yeast 10	Yeast 20	Yeast 40	SEM	P-value	R[1]
DM	73.12	74.14	70.53	73.46	1.434	0.311	
Crude protein	76.70^{ab}	76.12^{ab}	70.98^{a}	78.63^{b}	2.391	0.160	+
Starch	97.80^{ab}	97.96^{ab}	97.24^{a}	98.67^{b}	0.417	0.191	
Ash	39.91^{ab}	45.32^{a}	36.35^{b}	54.78^{c}	3.098	0.001	**++

[1]** = significant linear regression + = tendency quadratic regression ++ = significant quadratic regression
a, b, c – average values with different letters in the same row differ significantly at P≤0.05.
[2]Extreme outliers (>3*IQR from average) are excluded from the data.

No significant differences were found in AID of DM, CP or starch (Table 4.3). However, numerical values are showing poorer AID for all nutrients in piglets receiving the Yeast 20 diet (Table 4.3). AID of ash was higher (P<0.05) in piglets receiving the diet with the highest amount of yeast (Yeast 40). There was a positive correlation (P<0.05) between AID of ash and increasing levels of yeast. However due to the lower value of Yeast 20, the correlation also was quadratic (P<0.05). The same tendency (P=0.095) for a quadratic correlation was found in AID of CP. No effect of sex or litter was found on AID of main nutrients.

There were no significant effects of dietary treatment on AID of AA (Table 4.4). Numerically, the AID of all AA were lowest in the piglets fed Yeast 20, whereas the highest AID of AA were found in piglets fed the Yeast 40 diet and the control diet. No effect of sex was found on AID of AA. There was a significant effect of litter on AID of proline (P<0.05), but no effect on the other AA.

There were huge variation in some of the AA-results, thus the analyses will be repeated, but not in time to be included in this thesis.

Table 4.4 Effect of increasing levels of yeast in diets on AID of AA in weanling piglets.

	Dietary treatments[2]						
	Control	Yeast 10	Yeast 20	Yeast 40	SEM	P-value	R[1]
Indispensable AA							
Arginine	84.00	84.81	82.47	85.94	1.328	0.396	
Histidine	81.87	81.11	78.73	83.16	1.775	0.353	
Isoleucine	80.74	78.95	76.69	80.04	1.950	0.481	
Leucine	83.13	81.93	79.67	83.39	1.718	0.401	
Lysine	87.27^{ab}	87.14^{ab}	84.14^{a}	88.67^{b}	1.499	0.190	
Methionine	90.87^{ab}	90.55^{ab}	89.03^{a}	92.26^{b}	0.963	0.136	+
Phenylalanine	82.94	81.68	79.29	82.85	1.652	0.370	
Threonine	81.78^{a}	80.43^{ab}	76.77^{b}	78.20^{ab}	1.671	0.162	*#
Tryptophan	83.68	83.99	81.30	84.26	1.384	0.445	
Valine	81.41	80.14	77.08	80.74	1.800	0.354	
Dispensable AA							
Alanine	74.87^{ab}	74.35^{ab}	69.31^{a}	78.26^{b}	2.571	0.114	+
Aspartic acid	75.89^{ab}	74.96^{ab}	71.04^{a}	78.44^{b}	2.388	0.195	+
Cysteine	72.75	72.31	69.61	72.83	2.408	0.673	
Glutamic acid	85.95^{ab}	86.51^{ab}	82.43^{a}	87.47^{b}	1.543	0.119	
Glycine	60.29	56.01	50.47	61.49	6.160	0.647	
Proline	74.47^{ab}	78.60^{a}	68.58^{b}	74.11^{ab}	3.037	0.188	
Serine	79.12	77.77	74.47	77.46	1.890	0.390	
Tyrosine	71.68	70.81	66.10	71.79	2.838	0.432	

[1]* = tendency linear regression + = tendency quadratic regression # = tendency control vs yeast
a, b – average values with different letters in the same row differ significantly at P≤0.05.
[2]Extreme outliers (>3*IQR from average) are excluded from the data.

4.3.2 Apparent total tract digestibility coefficients (%).

Table 4.5 Effect of increasing levels of yeast in diets on ATTD of main nutrients in weanling piglets.

	Dietary treatments						
Nutrients	Control	Yeast 10[2]	Yeast 20	Yeast 40	SEM	P-value	R[1]
DM	83.23^{abc}	83.68^{ac}	82.55^{b}	83.82^{c}	0.299	0.020	
Crude protein	78.33^{ab}	80.18^{c}	77.82^{a}	79.93^{bc}	0.575	0.016	
NDF	36.13^{a}	33.71^{a}	22.47^{b}	25.30^{b}	2.063	<0.001	**##
Starch	99.67	99.71	99.66	99.67	0.031	0.758	
Ash	54.96^{a}	59.29^{bc}	53.33^{a}	59.54^{c}	0.898	<0.001	*##
Crude fat	71.00^{ab}	69.67^{a}	70.52^{ab}	74.23^{b}	1.351	0.077	*+
Energy, MJ/kg	82.42^{ab}	83.16^{a}	81.96^{b}	83.11^{a}	0.317	0.032	

[1]* = tendency linear regression ** = significant linear regression
+ = tendency quadratic regression ## = significant control vs yeast
a, b, c – average values with different letters in the same row differ significantly at P≤0.05.
[2] One pig was excluded from the dataset due to an unlikely high analyzed yttrium value in the feces (>3σ from mean value).

Dietary treatment significantly affected ATTD of main nutrients (P<0.05), except for crude fat (P=0.077) and starch. The intermediate yeast-diet (Yeast 20) stands out, with lower numeric ATTD values for all main nutrients, except for crude fat (Table 4.5). The highest numerical value for ATTD of DM was for Yeast 40. There were significantly higher ATTD of CP for Yeast 10 and a tendency for higher ATTD for Yeast 40 (P=0.055) compared to the control diet. Piglets receiving the Yeast 20 diet, had lower digestibility of CP (P<0.05) compared to the two other diets with yeast. The contrast analysis between the control and yeast-diets, are stating a significant effect of yeast inclusion on ATTD of NDF. There was a negative linear correlation (P<0.05) between ATTD of NDF and increasing levels of dietary yeast. The piglets fed control diet had (P<0.05) higher ATTD of NDF than piglets fed the diets with the two highest levels of yeast. However, the variation in NDF digestibility between individuals was large.

There was a tendency (P=0.059) for increasing ATTD of ash by increasing levels of yeast. The dietary treatments Yeast 10 and Yeast 40 had significantly higher digestibility of ash compared to the control diet (P<0.05). ATTD of crude fat was significantly higher in Yeast 40 compared to the Yeast 10, and tended to be higher than the control (P=0.096) and Yeast 20 (P=0.058).

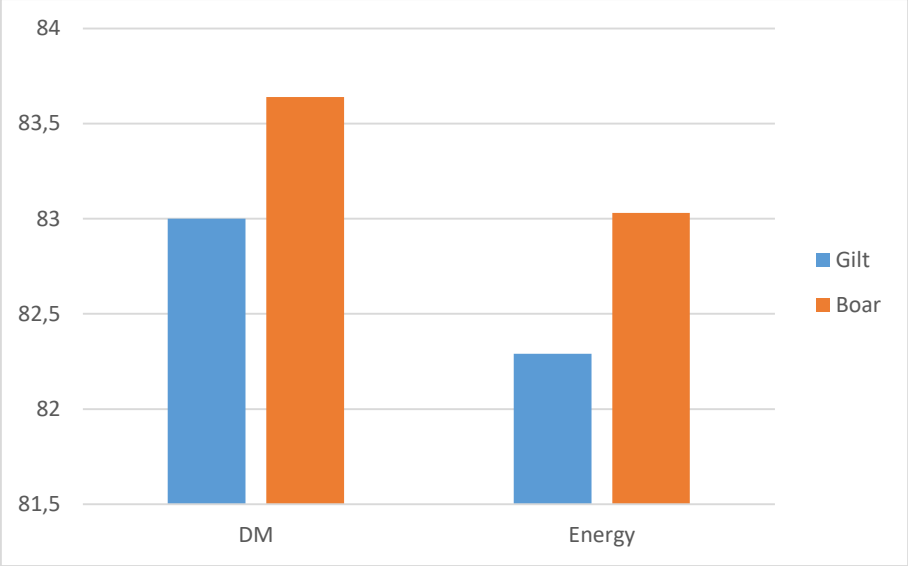


Figure 4.3 Effect of sex on apparent total tract digestibility coefficients (%) of DM and energy.

A correlation between litter and ATTD of DM (P=0.074), CP (P<0.05) and energy (0.078) was found. Boars had significantly (P<0.05) higher ATTD (83.03%) of energy than gilts (82.29%). There was also a tendency (P=0.070) for higher ATTD of DM in boars (83.64%) than gilts (83.00%) (Figure 4.3).

4.4 Health parameters

Some of the pigs suffered from diarrhea, mainly the second week of the experiment, and it appeared to be related to the stress around weaning. There were also some occurrences of diarrhea later in the experimental period. Day 6 to 8 some of the pigs vomited, but it was not related to dietary treatment.

One pig suffered from otohematoma otherwise no disease was recorded. None of the pigs received any medical treatment during the experiment.

4.4.1 DM in feces and fecal score

Table 4.6 Effect of increasing levels of yeast in the diets of weanling piglet on dry matter (%) in feces.

	Dietary treatments						
Day	Control	Yeast 10	Yeast 20	Yeast 40	SEM	P-value	R[1]
7	18.25[a]	22.44[ab]	24.12[bc]	27.70[c]	1.414	0.010	** ##
14	26.68	27.80	26.54	27.40	1.097	0.825	
21	27.12[a]	27.87[a]	27.30[a]	30.37[b]	0.649	0.026	** #

[1]** = significant linear regression # = tendency control vs yeast ## = significant control vs yeast
a, b, c – values in the same rows with different letters differ significantly at P≤0.05.

Day 7 there was a significant positive linear correlation (P<0.05) between dry matter in feces and increasing levels of dietary yeast. Day 14, the differences were equalized, but the same linear correlation (P<0.05) was seen on day 21 (Table 4.6), and in the five-day fecal samples for ATTD determination (P=0.064).

Table 4.7 Effect of increasing levels of yeast in the diets of weanling piglets on fecal scoring.

	Dietary treatments						
Week	Control	Yeast 10	Yeast 20	Yeast 40	SEM	P-value	R[1]
1	1.85	1.71	1.70	1.69	0.175	0.917	
2	2.65^{ab}	2.76^{a}	2.60^{ab}	2.39^{b}	0.126	0.219	*
3	2.35	1.94	2.13	2.29	0.146	0.214	+
4	2.35^{a}	2.12^{ab}	2.04^{bc}	1.79^{c}	0.107	0.005	** ##
1-4	2.45^{a}	2.27^{ab}	2.26^{ab}	2.15^{b}	0.076	0.059	**##

[1]* = tendency linear regression ** = significant linear regression
+ = tendency quadratic regression ## = significant control vs yeast
a, b, c – values in the same rows with different letters differ significantly at P≤0.05.

Fecal scoring results are showing a significant effect of inclusion of yeast for the 4-week experimental period. A significant linear correlation (P<0.05) was found in week 4 and for the overall period (Table 4.7), where the score is decreasing by increasing amounts of dietary yeast, as clearly shown in Figure 4.4

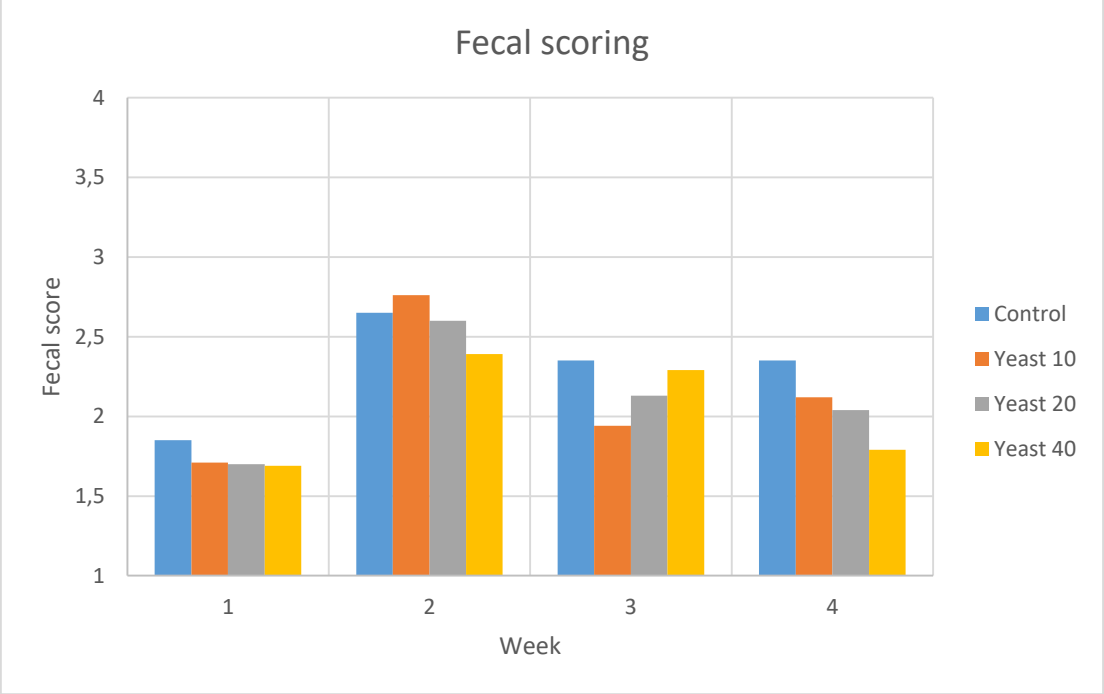


Figure 4.4 Graphic representation of average fecal score distributed by different dietary treatments and weeks of the experiment.

4.4.2 Hematology

Table 4.8 Effect of increasing levels of yeast in the diets of weanling piglet on hematology at day 7 and day 28 PW.

	Dietary treatments					
Hematologic test[1]	Control	Yeast 10	Yeast 20	Yeast 40	SEM	P-value
Day 7						
WBC ($x10^9$/L)	15.69	13.04	12.68	16.67	1.673	0.288
RBC ($x10^{12}$/L)	7.44	7.34	7.47	7.31	0.240	0.959
HGB (g/L)	128.86	131.60	120.80	128.17	4.347	0.410
HCT (L/L)	0.38	0.39	0.37	0.39	0.011	0.834
MCV (fL)	51.77	52.82	49.94	52.63	1.663	0.643
MCHC (%)	336.86	340.40	323.60	332.67	5.843	0.273
RDW (%)	20.74	18.36	21.70	21.77	1.550	0.432
PLT ($x10^9$/L)	456.71	348.20	449.20	452.00	57.444	0.525
NEUT ($x10^9$/L)	6.06	5.12	5.32	6.43	0.641	0.456
LYMPH ($x10^9$/L)	8.59	7.08	6.66	9.08	1.226	0.466
MONO ($x10^9$/L)	0.61^{ac}	0.44^{ab}	0.40^{b}	0.70^{c}	0.070	0.021
EOS ($x10^9$/L)	0.11	0.12	0.10	0.15	0.022	0.446
BASO ($x10^9$/L)	0.16	0.16	0.10	0.20	0.042	0.452
LUC ($x10^9$/L)	0.20	0.14	0.16	0.33	0.033	0.440
Day 28						
WBC ($x10^9$/L)	19.10			19.80	2.691	0.858
RBC ($x10^{12}$/L)	7.45			7.20	0.345	0.619
HGB (g/L)	123.20			119.50	3.092	0.420
HCT (L/L)	0.37			0.36	0.011	0.567
MCV (fL)	49.74			49.58	1.271	0.936
MCHC (%)	335.80			335.33	3.163	0.919
RDW (%)	19.50			19.83	1.159	0.844
PLT ($x10^9$/L)	495.80^{a}			389.83^{b}	26.580	0.020
NEUT ($x10^9$/L)	7.20			6.27	1.933	0.741
LYMPH ($x10^9$/L)	10.68			12.13	1.439	0.494
MONO ($x10^9$/L)	0.72			0.85	0.132	0.506
EOS ($x10^9$/L)	0.20			0.17	0.039	0.557
BASO ($x10^9$/L)	0.18			0.23	0.035	0.314
LUC ($x10^9$/L)	0.18			0.17	0.050	0.854

[1]WBC: white blood cell count; RBC: red blood cell count; HGB: hemoglobin; HCT: hematocrit; MCV: mean cell volume; MCHC: mean cell hemoglobin concentration; RDW: red blood cell distribution width; PLT: platelet; NEUT: neutrophils; LYMPH: lymphocytes; MONO: monocytes; EOS: eosinophils; BASO: basophiles; LUC: large unstained cells.

a, b, c – values in the same rows with different letters differ significantly at $P \leq 0.05$

Few significant differences in dietary treatments were found in hematology analysis at day 7 in this experiment. Mean values are shown in Table 4.8. However, there was a significant quadratic correlation in the absolute number of monocytes (MONO), with higher numbers for the control and Yeast 40. There was also a tendency for the same quadratic correlation on the count of white blood cells (WBC) ($P = 0.063$). The means of Yeast 10 and Yeast 20 for both WBC and MONO are being under the reference area (Sentrallaboratoriet 2010).

Day 28, there was a significant difference ($P<0.05$) in the number of thrombocytes (PLT) between the control and Yeast 40, with a higher number for the control treatment. However, both values are within the reference area (Sentrallaboratoriet 2010).

4.4.3 Liver index

Liver index was significantly affected by sex ($P<0.05$), with higher mean value for boars (3.12%) than gilts (2.93%). There was a tendency ($P=0.061$) of difference in liver index between diets, with the mean values for the control and Yeast 40 differing significantly. Whereas there was a significant linear increase ($P<0.05$) in liver index for increasing amounts of dietary yeast as shown in Figure 4.5.

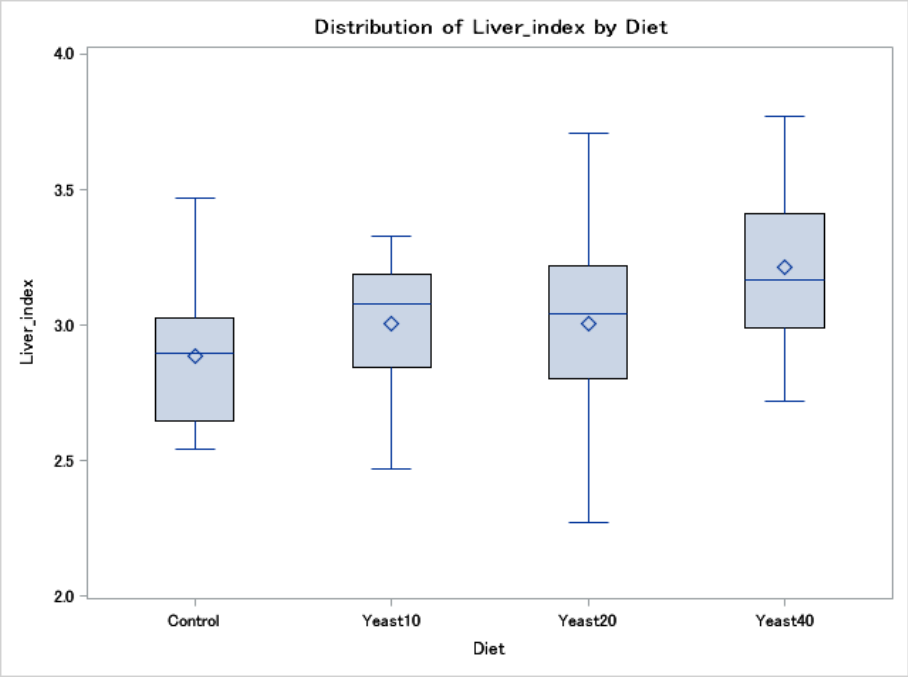


Figure 4.5 Distribution of liver index by dietary treatment.

5.0 Discussion

5.1 Performance

In the present experiment, four high quality protein sources were replaced in different amounts with *C. utilis* yeast. The diets were formulated such that the content of main nutrients were attempted to be similar between the diets. Chemical analyses of the diets showed more or less equal results for the four diets, with the largest difference in the content of NDF. However, it is not likely to think this will affect the piglets because of their young age and thereby limited utilization of NDF. There were also some differences in amino acid content, with lower values for some of the acids by increasing content of dietary yeast. Diets were formulated on the basis of similar crude protein content, but yeast contains more non-protein-nitrogen (nucleotides), thus whereas the calculated crude protein content from analysis of Kjeldahl-N is equal, the specific test for amino acids are showing a lower content.

In Norway, it is normal to add flavorings in the feed for piglets, to improve PW feed intake. Flavorings were not added to the experimental feed, on the purpose of not covering the palatability effect of the yeast. In addition, the products used for flavoring can contain substances that are covering the health effects of the yeast. Results did not show any significant effect of yeast inclusion on ADFI. However, in general, pigs receiving the diets with the highest levels of yeast (Yeast 20 and Yeast 40) ate more, indicating the feed with yeast was tasty. Similarly, Spark et al. (2005) reported increased feed intake PW by substitution of 40 % of soya protein with whey yeast (*Kluyveromyces fragilis*). Few studies are done with this amount of yeast in the feed for weanling piglets. Most previous studies with yeast have been testing it as a feed additive and not a feed ingredient as in this study. However, inclusion of yeast at a low level (≤ 6%) have been shown to give improved (Shen et al. 2009; White et al. 2002) or similar (Czech et al. 2016) feed intake as a control feed. Further palatability study with the yeast used in this experiment would be interesting to conduct.

The first week PW was of special interest in this experiment, as it is suggested in the literature (Cf. *2.4.3 Influence of yeast on the intestinal health*) that yeast may have a positive effect on the stress in the digestive system, resulted from the weaning and transition of feed. The growth decline often occurring in this period may have major consequences on the profitability in the production, as weight gain during first week post-weaning have major impact on subsequent growth performance (Kats et al. 1992). By inclusion of 4% yeast protein at the expense of fish meal, Hu et al. (2014) found that yeast inclusion had a positive effect on the ADG in the first

week PW. In the present experiment, a significant linear increase in ADG by increasing levels of yeast was found the first week PW, indicating inclusion of high amount of dietary yeast could reduce the growth decline often occurring. However, no differences were found in ADG during the four-week period PW, whereas Spark et al. (2005) reported increased ADG and improved FCR by substituting 40 % of the soy protein with whey yeast (*K. fragilis*), a side-product of cheese production. Similar, Hu et al. (2014) obtained a markedly higher overall ADG for a four-week period by inclusion of 4 % yeast-derived protein from baker's yeast, in diet fed to piglets weaned at 26 days of age, compared to a control feed. Increased ADG have also been found in older piglets (46-85 days of age), by inclusion of 3 % of *Y. lipolytica* yeast (Czech et al. 2016).

Even though the results of this study did not show any significant effect of dietary yeast on ADG of piglets during a four-week period PW, there was a significant difference in FCR between the diets. Including data from the first two weeks PW, there was a linear tendency for improved FCR by increasing levels of dietary yeast, although none of the means differed significantly. In the four-week PW period, greatest FCR was found in piglets fed Yeast 40, followed by the control feed, giving a significant quadratic correlation between dietary treatment and FCR. The intermediate yeast diet (Yeast 20) had significant poorer FCR compared to the other dietary treatments. The results indicate a positive effect of yeast inclusion on the utilization of the feed early PW, and no negative effect of 40 % yeast substitution in a four-week period PW.

The first week, numerical values are showing the greatest FCR for Yeast 20, however not significant. Including all the data in the analysis (appendix II), FCR for Yeast 20 actually is -1.57, due to huge weight losses the first weeks for some pigs. As some of the weight losses are considered very unlikely, data considered as extreme outliers, defined as 3*IQR from the means, are excluded from the dataset presented in main results. An explanation for the odd weight changes the first week may be due to the use of different scales, as there were used a different scale to determine the start weights at weaning than used in the rest of the experiment.

The hypothesis H1 predicts; "Inclusion of dietary yeast will result in improved performance of the piglets". Not enough support is found in the performance data of the present experiment to confirm this hypothesis. On the other hand, no negative effects of the Yeast 10 and Yeast 40 diets was found, which itself is a positive result, giving support to the main hypothesis, indicating that yeast may replace high quality protein sources in feed for weanling piglets.

Use of in-feed antibiotics has been forbidden for two decades in Norway, but in other part of the world use of AGP in the feed has been common. As mentioned in the literature review, inclusion of dietary yeast has been shown to be a suitable replacement for AGP (Maribo & Spring 2003; Shen et al. 2009; Van der Peet-Schwering et al. 2007; Waititu et al. 2016b). However, due to the general good infection- and hygienic conditions in pig production in Norway, performance results in general is good without the use of AGP. This may be a possible explanation for the lack of significant effect of dietary yeast inclusion on the performance results.

5.2 Digestibility

No significant effect of dietary treatment was found in AID of DM, CP or starch. However, ATTD of main nutrients was significantly affected by dietary treatment, except for crude fat and starch. Of the yeasts diets, ATTD of DM were significantly lower in the intermediate diet (Yeast 20). There were no effect of yeast inclusion compared to the control in ATTD of DM, and it differed from 82.55% to 83.82%. Spark et al. (2005) found higher ATTD of organic matter in diets with whey yeast compared to control without yeast supplementation. Kim et al. (2000) reported a 96.0 % ATTD of DM in Brewer's yeast (*S. cerevisiae*), 96.5 % in fishmeal, and 95.7 in SBM. Whereas Øverland et al. (2013), reported improved digestibility of protein in a diet with *C. utilis* compared to *S. cerevisiae* in Atlantic salmon. However, age of the piglets will influence the digestibility, as the maturation of the GIT largely takes place during the first two month post-partum (Manners 1976).

Dietary treatment significantly affected the digestibility of ash. Both AID and ATTD results showed an improved digestion and absorption of minerals by inclusion of dietary yeast. Yeast contains a lot of minerals with a high bioavailability. Higher phosphorous digestibility in yeast products from the ethanol industry and in brewer's yeast, compared to SBM are reported in growing boars by Kim et al. (2014). No data for different minerals are included in the present study, but good growth and health of the piglets indicate sufficient supply of essential minerals, and diets were formulated to cover the needs for the piglets.

There were small effects of dietary treatment on the AID of nutrients, but significant results for ATTD, which could be explained by an effect of the yeast on the large intestine microbiota. However, in that case it would be expected to find an improved NDF digestibility in piglets receiving yeast treatment, whereas the results are showing the opposite. By contrast, no effect

of inclusion of 0.75 % yeast culture on ATTD of NDF in weanling piglets was found by Kornegay et al. (1995). However, the study was aimed to test influence of yeast culture on different fiber sources and feed was given in meal form. In the present study NDF content differed between the dietary treatments, and the difference in digestibility could be a result of this. By contrast, Kornegay et al. (1995) reported decreased NDF digestibility by increased level of fiber in the diet, probably due to an increased rate of passage.

Digestibility of CP was not significantly affected by dietary treatment in the present study, ranging from 70.98 % (Yeast 20) to 78.63 % (Yeast 40) in AID, and 77.82 % (Yeast 20) to 80.18 % (Yeast 10) in ATTD. However, there were numerically improved AID of CP in the diet with the highest amount of yeast (Yeast 40). Whereas the ATTD of CP in the Yeast 10 diet was significantly greater than in the control diet, a numerically greater value was also the case for the Yeast 40 treatment. Spark et al. (2005) reported an increased ATTD of nitrogen (N) and N-retention by including increasing amounts of whey yeast (6 %, 12 % and 17 %) in the diets for reared piglets compared to a control SBM-feed. The CP digestibility was approximately 87 % in the whey yeast diets and 86.5 % in the control, some higher than in the present study, but the pigs was probably older, although the age of piglets in the experiment was not stated in the article. In Atlantic salmon, Øverland et al. (2013) reported 88 % digestibility of CP in a diet containing 28.3 % *C. utilis*, approximately the same as for a fishmeal diet. However, no SBM was present in the diets.

In the present experiment, AID of AA in general followed AID for CP. The piglets fed the Yeast 20 diet had numerically lower AID of all AA, whereas the piglets fed the Yeast 40 diet had numerically higher AID of most AA. Inclusion of yeast had no significant effect on AID of AA, indicating good digestibility of the yeast. Similar, in the mentioned experiment with Atlantic salmon by Øverland et al. (2013) they found no differences in apparent digestibility of AA between the fishmeal diet and the *C. utilis* diet. In the present experiment, AID of glycine was remarkably lower for all dietary treatments, corresponding to the results for cereal diets reported by Fan et al. (1994) and Sauer et al. (1991). This may be explained by a high concentration of glycine in endogenous secretions (Huang et al. 1999). Calculation of AID and ATTD does not consider endogenous losses and microbial metabolism, and may therefore underestimate the true digestibility of the CP and AA. Specific endogenous losses may be affected by concentration and type of fiber and antinutritional factors (Stein et al. 2007). The NDF content differed between the dietary treatment, which could have caused an

underestimation of the CP and AA digestibility in diets with higher NDF content, due to increased specific losses.

The results from the intermediate yeast diet (Yeast 20) differed unexpectedly from the pattern in the data. The pigs receiving Yeast 20 generally had poorer digestion of the main nutrients in the diet, except crude fat. In addition, the performance results are showing a higher FCR for this dietary treatment, corroborating the lower digestibility. As the piglets receiving Yeast 20 had the highest numerical feed intake, it is not likely that something was wrong with the palatability of the feed, nor with the general condition of the piglets as this would affect the feed intake. Since these piglets had the worse FCR, it suggests that something may have been wrong with the nutrient content in the feed. By studying the chemical analysis of the feed further, it appears that Yeast 20 had some lower content of crude protein and some higher content of starch than the other diets. In addition, analysis of marker content is showing a higher content of yttrium in this diet. A possible explanation for this result may be that there has been an error during feed production, resulting in this diet being different from the others. However, performance results are greater in the first weeks, when the piglets received the diet without digestibility marker; therefore, the error could be in the marker-feed. Looking for explanations, the poorer digestibility could be due to an increased temperature during feed production (Appendix I). The Maillard-reaction, where AA reacts with reducing sugars, may take place in room temperature, but is more frequent by increasing temperature. The reaction consequently decrease the availability, hence digestibility of AA in the feed (De Almeida 2013). Although a slightly higher temperature was measured in the Yeast 20 feed and the digestibility was decreased, no evidences for reduced AA availability due to Maillard-reaction is observed, and will only be guessing for an explanation. There is no clear reason why piglets fed this diet performed worse, and further discussion will only be guessing. Moreover, no support is found to claim this amount of yeast would lead to poorer performance of the weanling piglets.

5.3 Health

In general, the piglets seemed healthy during the experimental period. Some piglets suffered from PW diarrhea, but not as seriously that treatment was necessary. The hygienic conditions in general was good and the infection pressure relatively low. In Norway, there is a strict health monitoring. Some herds in Norway are defined as specific pathogen-free (SPF), meaning they are free for some defined pathogenic agents (viruses or bacteria), such as the respiratory pathogens *Actinobacillus pleuropneumoniae, Mycoplasma hyopneumoniae and Pasteurella*

multocida, the bacteria *Brachyspira hyodysenteriae* causing swine dysentery, and the itch mite *Sarcpotes scabiei* causing scabies (Hallenstvedt 2016). Consequently, this lowers the challenge levels for piglets in these herds. However, the focus on these pathogens leads to a lower infection pressure in the whole population. Even though the experimental piglets is not defined as a SPF-herd, these diseases are not observed in the current herd.

It was hypothesized that "Inclusion of dietary yeast in the feed may improve the intestinal health, and reduce the incidence of post-weaning diarrhea" (H3). No individual registration of incidences of severe diarrhea was made, so an indication of the occurrence is based on the fecal results. Feces DM samples from day seven showed significant differences where dry matter increased by increasing amount of dietary yeast, indicating the yeast might have a positive effect on the incident of PW diarrhea. The same linear correlation was also found at experimental day 21.

The fecal scoring was highest the second week for all dietary treatments. According to Madec et al. (1998), PW diarrhea often occurring around day 4-9 PW. The results of the present experiment showed a markedly increase in fecal score the second week for all dietary treatments. More specific, there was an increase in fecal score at day three and the highest scoring, with an average above 2.5 for all diets, was observed around day 7-10 (data not shown in this thesis). In accordance with Ball and Aherne (1987), a numerical decrease in fecal score from the second to the third week was observed in the present experiment, supporting their suggestion about a 2-week adaption period to solid food.

The significant difference in fecal DM day seven was not seen in the fecal scoring for the first week, although the differences in numeric values correspond. However, fecal DM samples were only collected one day each week hence dependent on the situation that particular day, and are therefore not representative for the whole week, whereas fecal scoring was done every day and is an average for the week. It should also be considered whether the samples were representative for the entire pen. As Pedersen and Toft (2011) are discussing, fecal scoring is not objective, and different persons doing the scoring may affect the results. Including use of different persons, several sources of errors are associated with the fecal scoring results. Fecal scoring was often done in the morning, including all feces from the night before. Water nozzles were present over the pens to promote the use of the slattered floor for feces to keep the resting area clean Because of this, including spilled water from the drinking nipples and urine mixed in the feces, an overestimation of the fecal consistency could be easily done.

The hypothesis (H1) “Inclusion of dietary yeast will result in improved performance of the piglets” and (H2) “Inclusion of dietary yeast will improve digestibility of the feed and metabolic utilization of nutrients”, is based on research showing improved intestinal health by inclusion of dietary yeast, as reviewed in the literature part of this thesis. By counteracting the changes in the intestinal morphology occurring during weaning, a better utilization of the feed is obtained; consequently improving the performance of the weanling piglet. In the present study, an improved apparent digestibility of some nutrients, a lower fecal score and higher fecal DM content for piglets fed dietary yeast, both the first week and the overall period, are indications of a better intestinal health, corresponding to some of the results in the literature.

Due to the change in feed, a new microbial community is re-established in the GIT after weaning (Heo et al. 2013). To promote a good environment and substrate access for the microbiota is important as they contributes in the fight against colonization of pathogenic bacteria in the GIT (Rist et al. 2013). However, it is only desirable to feed the beneficial microbiota, not the pathogenic. Low digestibility of nutrients in the small intestine will give more substrate for the microbiota to ferment, including the pathogenic, and lower digestibility is therefore associated with more diarrhea (Ball & Aherne 1987). Plant protein sources contain relatively high amounts of fermentable carbohydrates (Rist et al. 2013), and are in general less digestible than animal protein sources (Heo et al. 2013), consequently increasing the substrate access for the microbiota fermentation. Especially the fermentation of protein has been shown to not be beneficial, but rather increase the growth of pathogenic bacteria (Ball & Aherne 1987). Therefore, protein source, quality and level, hence the digestibility of the dietary protein, may influence the incidence of PWD (Rist et al. 2013). In the present experiment, the results for digestibility are indicating a greater fermentation in the large intestine in piglets receiving the intermediate yeast diet (Yeast 20), as the Yeast 20 dietary treatment had the lowest AID, and a higher ATTD relative to the AID. However, the ATTD for this dietary treatment was still poorer than for the other dietary treatments. The Yeast 40 diet had lower differences between AID and ATTD for CP, starch and ash, probably due to high digestibility of these nutrients in the proximal part of the intestine. However, no correlation between nutrient digestibility and fecal consistency was found in this study, as the diet with the lowest digestibility (Yeast 20) did not differ according to the fecal scoring and feces DM results.

Beneficial health effects of dietary yeast may be due to the content of nucleotides, as yeast contains a higher amount of nucleotides than the other protein sources in the diets. In milk, which is the main nutrient source of nursing piglets, nucleotides are natural components (Sauer et al. 2011). The weaning causes piglets to go through a period of physiological stress by change in diet, and often low feed intake. Nucleotides can be seen as "semi-essential" nutrients, and addition of dietary nucleotides may be beneficial for body function and health in such periods of nutrient sufficiency (Sauer et al. 2011). Martinez-Puig et al. (2007) reported reduced decrease in PW villous height and reduced incidence of diarrhea in weanling piglets fed dietary supplementation of nucleotides. In addition, SBM is known to contain antinutritional factors that may cause transient hypersensitivity (Heo et al. 2013). Consequently, replacing of soya protein with yeast may be beneficial.

The yeast cell wall is consisting of bioactive components, which may increase the piglets resistance to disease by a non-toxic stimulation of the immune system (Moran 2004). A small, but significant linear increase in liver index for increasing amounts of dietary yeast was found in the present experiment. Increased liver index by inclusion of dietary yeast is also reported in Atlantic salmon (Øverland et al. 2013). Organ indexes can be useful to identify organs where alterations caused by different treatments may occur. They can thus give indications about e.g., dietary imbalances or differences between dietary groups, but more in-depth analyzes need to be carried out to determine causes of such differences.

By dietary inclusion of mannan oligosaccharides, which also is present in the yeast cell wall, to weanling piglets, Zhao et al. (2012) reported improved ATTD of DM and nitrogen, improved performance results, and reduced diarrhea score. In similar, by supplementation of phosphorylated mannans derived from *S. cerevisiae* in feed for piglets weaned at 19 days of age, Davis et al. (2004) reported improved weight gain and feed efficiency. In addition, the immune system was intermittently affected both systemically and enterically.

In the present experiment, inclusion of dietary yeast had few or none effects on hematology results (blood composition), indicating that the general immune status of the piglet was not affected by yeast. However, although hematological parameters may be used to indicate general immune status, they should always be used together with other parameters such as immunoassays for detection of T and B cells, levels of immunoglobulins, expression of specific interleukins and many more. Neither, the hematology results are significantly different for the Yeast 20 diet; therefore cannot the health status of these piglets explain the poorer performance

results discussed earlier. The results corresponds to the results of Van der Peet-Schwering et al. (2007).

In the present experiment, all mean values for MCHC is in the upper layer or above the reference area (Sentrallaboratoriet 2010), indicating high concentrations of iron in erythrocytes. This is probably related to the iron supplement provided to all piglets by birth, and in the nursingperiod they had access to iron enriched peat (Normin® Ferro-Torv). At day seven, about half of the pigs had lymphocyte- and monocyte-count below the reference area. However, the reference area compared to is for older and heavier pigs, which could explain the deviating values.

Weaning age, breed, diet composition and sanitary conditions may affect the results of the present and other experiments, also suggested by Bontempo et al. (2006). As mentioned, the sanitary conditions of pig farms in Norway is in general very good, which may reduce the need for expression of beneficial health effect of the yeast. Beneficial effects of yeast may be expressed more clearly in an environment with a higher infection pressure. In addition, yeast strain, production conditions and processing, will affect the chemical composition and the biological availability of the yeast. Comparing three different forms of the *S. cerevisiae*, Jiang et al. (2015) found the greatest effects of live yeast inclusion on feed efficiency, intestinal development and systemic immunity. However, inclusion of superfine yeast powder also gave enhanced effects on the mentioned parameters, probably due to the small particle size, hence greater availability of the cell wall components, increasing the absorption and interaction with the enterocytes. The yeast included in the feed in the present experiment was dried and hence inactivated as a probiotic. Moreover, it consisted of whole cells, which may have prevented the availability of beneficial substrates, as it is reported the cell wall may be poorly digestible (Øverland & Skrede 2016). In addition, few studies are done with the *C. utilis* yeast in pigs, providing little direct comparison basis. More common is inclusion of the *S. cerevisiae* at a low level. However, in similar to Spark et al. (2005), the present experiment found no negative impact of dietary yeast inclusion in a high level on the observed health parameters (fecal consistency, liver index and blood composition) of weanling piglets.

6.0 Conclusion

The results of this study demonstrate that *Candida utilis* yeast produced from lignocellulosic biomass may be a suitable protein source in feed for weanling piglets. Apart from the discussed unexpected performance and digestibility results for the intermediate yeast diet, results are showing improved or similar digestibility and feed utilization of the diets containing yeast compared to the control. In addition, inclusion of dietary yeast also improved the fecal consistency, which is one of the most important parameters for post weaning health in piglets.

Future research

This thesis is a part of a larger experiment by Foods of Norway, a Centre for Research-based Innovation at the NMBU, with the aim to: 1. Evaluate if yeast can replace high-quality protein sources in diets for weanling piglets; 2. Investigate effects on feed intake and growth performance; 3. Evaluate the nutrient digestibility of diets containing increasing levels of yeast; 4. Assess the impact of the yeast on gastro-intestinal health and function of weanling piglets; 5. Examine whether yeast prevents rancidity of pig meat.

In the experiment, in addition to the presented results, intestinal segment tissue samples for histology, gene-expression, metabolomics and intestine wall enzymes were taken from jejunum, ileum, cecum and colon. Intestinal content samples from the same segments were collected for measuring of pH, microbiota, metabolomics and enzymes. Organ samples were taken from liver, kidney, ileal lymph nodes and spleen. Blood was sampled for flow cytometry, metabolomics and gene expression, urine for metabolomics, and *Longissimus dorsi* was sampled for product quality.

In addition, at the day zero, seven littermate pigs of similar body weight were sampled to provide a baseline time point. Intestinal digesta and intestinal tissues from the different segments were also sampled for microbiota analyses.

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Appendix I

Data from feed production and pellet quality analyses.

	Dietary treatments			
Pelleting process	Control	Yeast 10	Yeast 20	Yeast 40
Conditioner temp, ºC	74.0	75.0	74.8	62.5
Motor load p.press, %	37.5	37.5	39.5	46.0
Power consumption, amp	34.0	34.0	34.5	38.0
Steam pressure, bar	2.5	2.5	2.5	2.5
Steam consumption, kg/h	38	38.5	34.9	31
Pellet temperature, ºC	91.6	90.1	93.1	82.7
Pellet quality analysis				
Pellet durability index	96.2	97.0	97.6	97.9
Moisture content, %	13.00	13.06	12.79	12.17

Appendix II

Performance results including data for all individual piglets.

Week		Dietary treatments				SEM	P-value	R[1]
		Control	Yeast 10	Yeast 20	Yeast 40			
0-1	ADG g/d	100	104	98	168	26.8	0.212	
	ADFI g/d	202	182	213	206	9.8	0.173	
	FCR	2.74	2.50	-1.57	1.34	1.099	0.046	*
0-2	ADG g/d	181	175	208	188	12.9	0.295	
	ADFI g/d	275	263	294	281	11.2	0.268	
	FCR	1.59	1.53	1.49	1.65	0.140	0.856	
0-3	ADG g/d	269	265	295	285	11.4	0.223	
	ADFI g/d	368	361	404	377	12.2	0.095	
	FCR	1.38	1.37	1.38	1.33	0.029	0.580	
0-4	ADG g/d	334	328	329	350	12.5	0.555	
	ADFI g/d	457	457	496	465	13.1	0.132	
	FCR	1.38	1.41	1.52	1.34	0.033	0.004	++

[1]* = tendency linear regression ++= significant quadratic regression

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