The Influence of Effective Micro-organisms (EM) on the Performance of the Growing Pig

By

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Chapter 1 A review on probiotics with special emphasis on pigs

Abstract

This review examines probiotics as a possible alternative growth promoter. Probiotics can be defined as a live microbial feed supplement, which affects the host animal beneficially, by improving its intestinal microbial balance. Nearly all the probiotics on the market contain lactobacilli and/ or streptococci and to a lesser extent bifidobacteria. If probiotic organisms are to survive and be active in the digestive tract, they have to be suitable for that environment and resist the hosts' protective mechanisms, which are inhibitory to microbes. The form of presentation of the microorganisms as probiotics is largely dependent on convenience for distribution and administration, provided essential characteristics are maintained. Microbes must be metabolically active for effective action. Thus, the form of presentation must ensure the viability of the microbes. If the hosts' body is already functioning optimally, animals will most likely be growing at their maximum capacity and therefore it may not be possible to achieve any improvement by the administering of beneficial bacteria. However, stress conditions may affect the balance of the gut micro flora adversely. This is situation in which probiotics may be of potential value. The restoration of the gut flora will enable the host animal to return to a normal production level.

Samevatting

Hierdie oorsig evalueer probiotika as 'n moontlike alternatiewe groei promotor. Probiotika kan gedefinieer word as die supplementering van lewendige mikroorganismes wat die gasheer dier voordelig beinvloed deur die balansering van die die dier se spysverteringskanaalmikrobes. Die meeste probiotika beskikbaar op die mark bevat lactobacillus en/of streptococci en tot 'n mindere mate bifidobakterium. Vir probiotika-organismes om te oorleef en aktief te funksioneer in die spysverterings kanaal moet die organismes geskik wees vir hierdie omgewing en moet hulle

daartoe instaat wees om die gasheer dier se natuurlike weerstandsmeganismes te oorkom wat oor die algemeen mikrobegroei inhibeer. Die vorm waarin probiotika versprei en toegedien kan word gaan hoofsaaklik bepaal word deur die gerief daarvan, met die voorbehoud dat alle essensiële eienskappe van die produk behou word. Dus die vorm van verspreiding moet die lewensvatbaarheid van die organismes verseker. Indien die gasheer dier se liggaam reeds optimaal funksioneer, sal die diere in die meeste gevalle reeds hulle maksimum groeikapasiteit bereik het en sal die toediening van probiotika nie groei verder verbeter nie. Toestande van stres beinvloed egter die balans van mikrobes in die spysverteringskanaal nadelig. Dit is wanneer hierdie toestande heers dat probiotika tot sy reg kom. Deur die spysverteringskanaalmikro-organismes te balanseer stel dit die dier instaat om na sy normale vlak van produksie terug te keer.

Key words: Probiotics, balance, gut flora.
Sleutel woord: Probiotika, balans, spysverterings kanaal mikrobes.

Chapter 2 — The influence of Effective Microorganisms (EM) on the growth performance of the pig

Abstract

Forty-five weanling pigs were used to examine the effect of Effective Microorganisms (EM) and a commercial antibiotic on the growth performance and backfat thickness of the growing-finishing pig. Dietary treatments were EM Bokhasi at inclucion levels of 1% EM Bokhasi, 2% EM Bokhasi, 3% EM Bokhasi, the antibiotic and the control treatments. The overall average daily gain (Weeks 1 to 12), mean body weight gain and feed conversion ratio were not significantly influenced by the different treatments. However, EM Bokhasi at 2 % and 3 % inclusion levels had a significant ($P<0.05$) lower mean feed intake than the commercial antibiotic and during weeks one to three the antibiotic had a higher ($P<0.05$) average daily gain than the EM 2% and 3%

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treatments. No significant results between the different treatments were recorded for the backfat thickness. However, there was a tendency for EM Bokhasi at an inclusion level of 1% to reduce backfat thickness. In summary, the inclusion of EM Bokhasi and an commercial antibiotic in the diet of growing-finishing pigs did not improve average daily gain, mean body weight gain feed conversion ratio and backfat thickness significantly. The inclusion of a commercial antibiotic did improve the mean feed intake and average daily gain during, weeks 1 to 3, in comparison with the EM Bokhasi treatments with inclusion levels of 2 and 3 %.

Samevatting

Vyf en veertig speenvarke is gebruik om die invloed van Effektiewe Mikroorganismes (EM) en >n kommersiële antibiotika op die groeiprestasie en rugvetdikte van die groeiende varke te bepaal. Die verskillende behandelings was; die insluiting van EM Bokashi teen vlakke van 1, 2 en 3% in die dieet, die kommersiële antibiotika en die kontrole dieet. Die verskillende behandelings het geen effek gehad op die algehele gemiddelde daaglikse toename (weke 1 to 12), gemiddelde liggaamsmassatoename en voeromsetverhouding nie. Die antibiotika het egter 'n betekenisvolle (P<0.05) hoër gemiddelde voerinname gehad as die EM Bokhasi 2 en 3 % behandelings. Die laasgenoemde twee behandeligs het ook 'n laer (P<0.05) gemiddelde daaglikse massatoename vir weke 1 tot 3 gehad as die antibiotikabehandeling. Geen betekenisvolle resultate is verkry vir die rugvetdikte nie, maar daar was wel 'n duidelike tendens vir die EM bokhasi 1% behandeling om rugvetdikte te verlaag. Om op te som; die insluiting van EM Bokhasi en 'n kommersiële antibiotika het geen invloed gehad op die gemiddelde daaglikse massatoename, gemiddelde liggaamsmassatoename, gemiddelde voeriname, voeromsetverhouding en rugvetdikte nie. Die antibiotika het egter die gemiddelde voerinname en die gemiddelde daaglikse massatoename van weke een tot drie betekenivol (P<0.05) verhoog bo die EM Bokhasi 2 en 3 % behandelings.

Key Words: EM Bokhasi, growth performance, pigs

Sleutel woorde: EM Bokhasi, groei, varke

Chapter 3 The influence of Effective Microorganisms (EM) on nutrient digestibilities of growing pigs

Abstract

Thirty barrows with an average body weight of approximately 55 kg were used in a conventional digestibility experiment to investigate the influence of the dietary addition of different levels of EM Bokhasi and a commercial antibiotic on nutrient digestibility and N retention. The different treatments were as follows: EM Bokhasi at inclusion level of 1, 2 and 3 %, a commercial antibiotic and control treatment. The EM 2 % treatment had a significantly ($P<0.05$) higher ME MJ/kg value, ME/DE % and N balance as % of N intake than the control treatment. The control treatment had a lower ($P<0.05$) N digestibility coefficient and ether extract digestibility than the EM 1 %, EM 2 % and antibiotic treatments. The EM 2 % treatment had a higher ($P<0.05$) dry matter digestibility coefficient, apparent digestible energy % and ME MJ/kg than the EM 3 % treatment. On the other hand EM 3 % treatment had a significantly lower dry matter digestibility coefficient and N digestibility coefficient than the antibiotic and a significantly ($P<0.05$) lower ether extract digestibility coefficient than the EM 1 %, EM 2 % and antibiotic treatments. The antibiotic had a significant ($P<0.05$) higher crude fibre digestibility coefficient than both the EM 2 % and EM 3 % treatments. The ash absorbed % of the EM 1 % treatment were a lower ($P<0.05$) than that of the EM 2 %, antibiotic and control treatments.

Samevatting

Dertig gekastreede bere met 'n gemiddelde liggaamsmassa vam ongeveer 55 kg is gebruik in 'n verteringstudie om die invloed van die toevoeging van verskillende vlakke van EM Bokhasi en 'n

kommersiële antibiotika op nutriëntverteerbaarheid en N- retensie te bepaal. Die verskillende behandelings het bestaan uit die insluiting van EM Bokhasi teen vlakke van 1, 2 en 3 % en antibiotika in die basale dieet. Die EM 2 % behandeling het 'n betekenisvolle hoër ME MJ/kg, ME/VE % en N balans as % van N inname gehad as die kontrole behandeling. Die kontrole behandeling daarinteen het ook betekenisvolle (P<0.05) laer N en eterekstrakverteerbaarheid koëffisiënte as die EM 1 %, EM 2 % en atibiotika gehad. Die EM 3 % behandeling het 'n laer (P<0.05) droëmateriaalverteerbaarheids koëffisiënt, skynbareverteerbare-energiekoëffisiënt en ME MJ/kg gehad as die EM 2 % behandeling en ook betekenisvolle laer droëmateriaal en N verteerbaarheids koëffisiënte as die antibiotika. Die eterekstrakverteerbaarheids koëffisiënt van die EM 3 % behandeling was ook laer (P<0.05) as die van die EM 1 %, Em 2 % en antibiotika behandelings. Die antibiotika behandeling het ook 'n betekeisvolle hoër ru-veselverteerbaarheids koëffisiënt gehad as die EM 2 % en EM 3 % behandelings. Die EM 1 % behandeling het egter ook 'n laer (p<0.05) as absorbeerbaarheids % gehad as die EM 2 %, antibiotika en kontrole dieet.

Key words; EM, digestibility, pigs.
Sleutel woorde; EM, verteerbaarheid, varke

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Chapter 1 A review on probiotics with special emphasis on pigs

Introduction

Pig production has become more industrialized with intensive and semi - intensive commercial units. The risk of major economic losses due to decreased performance and health has become of paramount importance and massive efforts have been made to find different ways to improve production. Medical products such as antibiotics and chemotherapeutics have been used very successfully against the decrease in growth performance (Jonsson & Conway, 1992).

Antibiotics as feed additive for farm animals have been used extensively since their discovery and the ready availability after the Second World War. The main rationale behind their ever-increasing use has been the clear demonstration that both growth and health of livestock improve when animals are fed on diets supplemented with various antibiotics (Puszatia et al., 1990). However, consumers are becoming increasingly concerned about drug residues in agricultural products including meat. In addition, it has been suggested that the continuous use of antibiotics may contribute to a reservoir of drug resistant bacteria, which may be capable of transferring their resistance to pathogenic bacteria in both animals and humans. Thacker (1988) speculated that the future use of antibiotics in animal feed may be restricted, and this is becoming an increasing probability.

Alternative methods to antibiotics must be developed that will improve animal performance in order to allow continued development of the pig industry. Probiotics may have the potential to become part of these alternative methods of improving growth. Probiotics have the opposite effect to antibiotics on the microorganisms in the digestive tract. Where antibiotics control the microbial population in the intestine by inhibiting or destroying microorganisms, probiotics actually introduce live bacteria into the intestinal tract in addition to the resident microbes (Thacker 1988). The purpose of this review is to take a look at the possible future role of

probiotics in pig nutrition.

Definition

The word "Probiotic" is derived from the Greek meaning "for life" and had several different meanings over the years. It was first defined as a substance secreted by one microorganism, which stimulated the growth of another, meaning exactly the opposite of "antibiotic". Thereafter it was defined as tissue extracts that stimulated microbial growth Fuller (1992a). In 1974 Parker, as cited by Fuller (1992b), defined the word "Probiotics"as an organism or substance which contributes to the intestinal microbial balance. This definition however, was too comprehensive and the word "probiotic" was again redefined according to Fuller, (1989) as "A live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance".

Composition of probiotics

Probiotic preparations may consist of a single or may contain any number of strains. Nearly all the probiotics on the market contain lactobacilli and/ or streptococci and to a lesser extent bifidobacteria. Currently the most widely used species in the preparation of probotics are *Lactobacillus bulgaricus, L. casei, L. lactis, L. salivarius, L. plantarum, Streptococcus thermophilus S. lactis, S. cremoris, S. diacetilactis, S. intermedius, Enterococcus faecium, Ent. faecalis, Bifidobaterium adolescentis, Bif. Animalis, Bif. Bifidum, Bif infants, Bif. Longum, Bif thermophilum. and E. coli.* Probiotics may also contain bacteria belonging to the genera *Leuconostoc, Pediococcus, Propionibacterium* and Bacillus yeasts and moulds. All of these strains are intestinal strains, except for L. *bulgaricus* and *Strep. termophilus,* (Fuller 1989; Fuller 1992a).

Lactic acid bacteria comprise a wide range of genera including a considerable number of species, all of which are able to ferment carbohydrates to lactic acid as the major end product. They are typically Gram-positive, usually catalase-negative, anaerobic and non-sporeforming bacteria (Klein 1997). The most important genera of lactic acid bacteria are *Lactobacillus, Lactococcus, Enterococcus, Sterptococcus, Pedicoccus, Leunnconostoc, Weisella, Carnobacterium, Tetragenococcus* and *Bifidobacterium*. However, the strain used most often as probiotics, according to Klein (1997), belongs to the genera *Lactobacillus, Enterococcus* and *Bifidobacterium*. Lactobacilli are seldom pathogenic (Jonsson & Conway, 1992). However, some strains of *Ent. faecalis* and *Ent. faecium* has been found to be pathogenic.

Bifidobacteria are more oxygen sensitive than most lactobacilli, and some strains are strictly anaerobic. Consequently, their survival in air will be more of a problem than for the other Lactobacilli used (Jonsson & Conway, 1992). On the other hand, streptococci are more tolerant to harsh conditions and therefore would yield more stable preparations than those containing Lactobacilli. Bacillus spp. on the other hand are mostly soil organisms which are used for the production of antibiotic substances. It is not known if the Bacillus spp. constitute a component of the indigenous micro flora or whether they are transient in the digestive tract. It has been claimed that the spores would germinate in the anterior part of the digestive tract and compete with enterotoxigenic *E. coli*. They may increase the Lactobacillus flora or stimulate the immune system against *E. coli*. It is feasible that performed antibiotic substances could mediate their action especially if they are given as spores in their propagation medium (Pollmann, 1986; Jonsson & Conway, 1992).

Mode of action

If ingested probiotic organisms are to survive and be active in the digestive tract, they would have to be suitable for that environment and resist the host's protective mechanisms, which are inhibitory to microbes. Inhibitors are the gastric juices, which contain mucus which have a low

pH due to the secretion of HCl and have active proteolytic enzymes. The pH of the stomach may vary between 2.6 and 4.9 for the weaned piglet and between 2.3 and 4.5 in the adult pig. The retention time as well as the degree of mixing of the ingested material with the gastric juices and previously indigested food also influences survival of the administered microorganisms.

The most important defense in the anterior part of the small intestine is the very fast flow rate, which prevents microbial overgrowth unless the microorganisms can be attached to the epithelium in this site. Among other factors, the presence of bile in this region also negatively influences the survival and activity of the microbes. A relatively rapid transit time in the posterior small intestines also protects the host unless the invading microbes can adhere to the epithelial mucosa. In the caecum and large intestine the passage rate is lower and the microbes can become established. However, they have to compete with a stable indigenous micro flora in the healthy host. The extent of survival in the stomach, together with the volume of the digesta found in the different parts of the digestive tract, influence the numbers of the probiotic organisms required for dosage (Jonsson & Conway 1992).

The beneficial effects of probiotics may be mediated by a direct antagonistic effect against specific groups of micro organisms, resulting in a decrease in numbers or by an effect on their metabolism, or by stimulation of immunity. According to Fuller (1989), experimental data support some of these purposed mechanisms, although not consistent for all of the mechanisms.

Pollman (1986) found that the beneficial actions of probiotics may also be mediated by a change in the enteric flora and reduction of *E. coli*, the synthesis of lactate with subsequent reduction in intestinal pH, adhesion and colonization in the digestive tract, production of antibiotic substances and the reduction of toxic amines and ammonia levels in the gastrointestinal tract and blood (Sissons,1989). Some strains also may have the ability to produce hydrogen peroxide, which may have antibacterial effects (Jonsson et al., 1985; Thacker, 1988; Sissons, 1989). Fuller (1989) suggested that the mode of action of probiotics may also include in addition to the above-mentioned actions, competition between probiotic organisms and the micro flora of the digestive

tract, competition for adhesion sites, alteration of microbial metabolism by both an increase or decrease in enzyme activity and the stimulation of immunity by both an increase in antibody levels and macrophage activity.

Another approach may be to use competitive colonization whereby nonpathogenic strains are given in order to prevent subsequent colonization by pathogenic strains. As with vaccinations, this approach will only protect the host from very closely related strains to that administered. The mode of action is solely to compete for same sites within the gut epithelial surface (Jonsson & Conway, 1992).

Fuller (1989) accentuated the fact that adhesion of microorganisms in the digestive tract is a host specific phenomenon and that adhesion varies between strains of the same species. The survival of probiotic organisms in the gut depends a great deal on their ability to colonize the gut which would enable them to resist the antibacterial mechanisms (both chemical and physical) operating in the digestive tract. The results of Jonsson et al. (1985) showed that no permanent establishment of any administered strain of Lactobacillus could be detected. In contrast Pollmann et al., (1980b) found that Lactobacillus in fact did colonize in the gut after administration and that the Lactobacillus populations increased with increased age of the pigs in their study.

According to Perdigon et al., (1995) the addition of *Lactobacillus casei* to the diets of malnourished animals prevented enteric infections and stimulated secretory IgA. Yogurt could inhibit the growth of intestinal carcinomas through increased activity of IgA, T cells and macrophages. According to Fuller, (1989) in order for the microorganisms to have these kinds of systemic effects it may be necessary for them to migrate from the gut to the systemic circulation. Lactobacillus can translocate (Fuller 1989) and can survive in the spleen, liver and lungs for many days (Perdigon et al., 1995).

The mode of action of probiotics may not always be beneficial to the host animal. According to Pollman (1986) there is speculation that mode of action of probiotics also may have some

negative effects on pig performance by competing for nutrients with the indigenous organisms of the digestive tract, decreasing carbohydrate utilization and by increasing the transit rate of digesta.

Indigenous gut micro flora

A relatively rich micro flora colonizes the foregut (stomach and small intestine) of the pig. The relative proportion of the microorganisms present vary in different parts of the gut. In healthy pigs, lactobacillus and streptococcus species are often the predominant species in the stomach and small intestines. They are found in both the digesta and attached to the epithelia. Bacteroides are normally absent from these sections of the gut, although the anaerobe *Veillonella* is present in small numbers. In the large intestine, the number of Lactobacili and Bacterroides present are broadly comparable. The predominant organisms in the caeca of normal pigs are *Bacteroides (Prevotella) ruminicola* and *Selenomonas ruminantium*. Apart from *Lactobacillus acidophilus* and *Butyrivibrio,* no other species contribute significantly to the numbers of microorganisms in this part of the gut. The colon epithelium of normal pigs is colonized mainly by Streptococci and bacteroides and apart from *Lactobacillus acidophilus,* no other species contribute significantly to the numbers of microorganisms in the colon (Stewart et al., 1993).

However, according to Jonsson & Conway (1992) the type and amount of diet have been shown to affect the lactobacilli in the digestive tract. Lactobacilli are more affected than *E. coli* and other pathogens, by a lack of nutrients. When pigs were deprived of food and water it was mainly the numbers of lactobacillus and bifidobacteria in the foregut and ileum that decreased, while the number of E.coli and Bacteroides increased.

Influence on the digestive tract and micro flora

In common with other species, the alimentary tract of the porcine fetus is sterile until birth.

Thereafter contact with the sow and the environment leads to the colonization by a variety of microorganisms. This establishment of bacterial activity is regarded as complementary to the digestive functions of the host by extending the range of digestive enzymes and under normal conditions as a barrier against invading pathogens. At times of stress the balance of the intestinal micro flora may become disturbed and disorders in the digestive function are likely to occur (Sissons, 1989).

The main reason for using probiotics is to stabilize the digestive micro flora and to compete with pathogenic microbes within the digestive tract. As already mentioned, Lactobacilli are strong acid producers which contribute to the lowered pH and decrease in number of bacteria entering the small intestines. In the neonatal pig half of the lactose in sows' milk is metabolized to yield organic acids in the stomach (Jonsson & Conway 1992).

The presence of microorganisms causes many changes in the physiology and morphology of the digestive tract of the host animal. The digestive tract of gnotobiotic animals has more slender and finger villi, thinner small intestines with reduced *lamina propria*, longer cell renewal time and higher digestive enzyme activities (Jonsson & Conway 1992).

Once the complex micro flora in the digestive tract develops, the system is relatively stable. Addition of further microbes to the stable indigenous micro flora should not give any changes in micro flora if the other conditions remain the same. However, although the microflora population is stable, it is also dynamic. Strains will replace each other and the metabolism will adapt itself to the available substrates. The addition of small numbers of bacteria or their metabolites into the large intestine could have substantial influence on the micro flora due to the competition for limiting nutrients in this part of the digestive tract. Studies showed that only 10 cells of *Salmonella enteritidis* are necessary to kill gnotobiotic mice, but it requires 10^6 cells to kill a conventional mouse. This may support the claim that the oral administration of sub-therapeutic levels of antibiotics, infections of the intestines may induce, which may result in enteritis or diarrhoea. It is proposed that the administration of sub-therapeutic levels of antibiotics the

protective indigenous microflora suppresses which allows the more resistant pathogenic species to increase (Fuller 1989). On the other hand the administration of antibiotics may aid the colonization of the gut by probiotic organisms. This may be a useful way of preparing the gut to accept probiotic strains.

Jonsson & Conway (1992) summarizes the results obtained from studies to determine the effect of the administration of probiotics on the gut flora as follows, the administration of probiotics might in many instances, influence the microbial flora in the digestive tract, especially when the indigenous micro flora has been disturbed. This balancing of the micro flora is difficult to analyze and may not always be connected with improvements in health or performance. However, should this stabilizing effects do not occur, it is possible that detrimental effects on health and performance could well be demonstrated@.

Use of probiotics

Probiotics can be presented to the animal in various ways. The type of preparation will depend on the sort of use intended. They can be included in either the pelleted feed or produced in the form of capsules, paste, powder or granules which can be used for dosing the animals directly through their food (Fuller 1989) or as viable organisms in wet, frozen or freeze-dried preparations, or as fermentation products with or without inactive organisms (Jonsson & Conway 1992). Probiotic preparations can be given soon after birth, at times when the farmer expects diseases (preventive or curative), or mixed into the food for continuous supply. The microorganisms can be injected orally or distributed in the water and/or feed.

The form of presentation of the microorganisms as probiotics is largely dependent on convenience for distribution and administration, provided essential characteristics are maintained. Microbes must be metabolically active for effective action. Thus, the form of presentation must insure the viability of the microbes. Probiotics may be given at different ages of the pig depending on the

proposed mechanism of function. If there is reason to believe that the normal indigenous micro flora of the healthy pig will not be established, preparations containing solely Lactobacillis are probably most desirable. On farms with a high incidence of diarrhoeal disease, it may be appropriate to introduce a probiotic strain as early as possible and thereby colonize the digestive tract with the probiotic strains that have the ability to inhibit pathogens.

The question may be asked whether single or a continuous dosage should be used. If the desired strain lacks the capacity to colonize the digestive tract continuous or daily dosages would be required. But, if the probiotic strain has the characteristics, which facilitate colonization of the digestive tract, only a single dosage is necessary until such time as the pig is exposed to some form of stress (Jonsson & Conway 1992; Sainsbury 1993).

Characteristics of a good probiotic

To exert any beneficial effect, the bacteria have to reach the site of function. In order to do so, first of all they have to be consumed. This means that the preparations have to be appetizing or at least not repulsive to the pig. Pigs are sensitive to the long- term effects as well as the flavour and taste of their feed (Jonsson & Conway 1992).

Use of probiotics often gives inconclusive or conflicting results (Jonsson et al. 1985; Pollmann 1986; Thacker 1988; Fuller 1989; Jonsson & Conway 1992). Strains used in probiotics can vary in their characteristic and thus their actions. If the host' s body is already functioning optimally, animals most likely will be growing at their maximum capacity and therefore it may not be possible to achieve any improvement by administering beneficial bacteria (Jonsson & Conway 1992). According to Fuller (1989) probiotics are bound to be variable because they operate by reversing stress factors, which may or may not be present. This is particularly likely in the case when it is being used as a growth promotor and the organism responsible for the growth depression is not present in the gut. On the other hand, this also occurs with antibiotics and other

chemical growth prompters when used under these circumstances. The practical consequence is that probiotics may work on one farm but not on the next farm and on one occasion but not the next. This is a major limiting factor. Some possible reasons for this variability of results are that the viability of microbial culture which may be dependent on storage method and cryoprotective agents in freezing technique, strain differences, dose level and frequency of feeding of the culture, species specificity problems, drug interactions and a lack of systematic investigation by researchers (Pollmann, 1986). Other factors like temperature, change in pH and various antibiotics are also known to decrease the viability of Lactobacillus cultures (Thacker, 1988).

The characteristics of a good probiotic are: it should contain viable cells of the species specified on the label; it should be non pathogenic and not have adverse effects of any sort; it should have a beneficial effect on the host animal; it must be able to survive and metabolize in the intestine (Jonsson et al., 1985); it must be resistant to a low pH; they should be good colonizers and inhibitors; it should be tolerant to bile, it should be of a good quality; it should remain stable in storage conditions (Fuller, 1989; Sissons, 1989; Gadd, 1990). A probiotic with all the above-mentioned features has considerable advantages over antibacterial supplements. Probiotics do not induce resistance to antibiotics, have no side effects, leave no residues in carcasses and they may stimulate immunity, where as the immune status remains unaffected by antibiotics (Fuller 1989).

Influence of probiotics on performance

When the piglet is born, it may get a serious challenge from pathogenic organisms. In the wild the baby animal picks up its gut flora mainly from its mother by direct or indirect routes. Under the conditions of modern animal husbandry stress factors are induced which may interfere with the normal establishment of the gut flora. These stressors may include the following: temperature; stress; dehydration; injections causing immunological and irritant stress; poor supply of feed and water; infections; poor ventilation; parasites infestations; the feeding of antibiotics and growth promoters (Sainsbury 1993). During these stress conditions the general trend is for Lactobacilli

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to decrease and the coliforms to increase. Hormonal changes induced by the stress may also affect the production of mucus which may in turn reduce the components of the gut flora which are normally associated with it. These conditions, where the balance of the gut micro flora is adversely affected, are all situations in which probiotics are of potential value. The restoration of the gut flora will enable the host animal to return to normal (Fuller 1989).

A major aim with using probiotics is to improve the performance and health of the animals. Growth rates, feed utilization, number of deaths and days with diarrhoea, sometimes irrespective of cause, are the most common parameters that need to be improved by probiotics. As previously mentioned results are very conflicting.

It has been reported in many trails that Lactobacillis tends to improve performance and health. Suckling piglets with chronic diarrhoea problems showed improvements after the administration of *L. acidophilus* strains. In some trails, starter pigs given *L. acidophilus* have shown improved average daily gain and feed conversion. However, the performance of growing and finishing pigs have not been influenced. The greater part of the experiments reported, showed positive effects on health and growth after the administration of Streptococci especially the *Ent. faecium* strains. In contrast higher mortality rates were reported for pigs that received the *E. Coli* M74 strains.

Under suboptimal conditions improvements of performance and health were shown. The same results were not obtained when pigs were raised under good hygienic conditions. Sows treated with *Bacillus toyoi* had lower frequencies and milder symptoms of the mastitis-metritis-agalactia syndrome (Jonsson & Conway 1992). According to Nguyen (1991) the addition of probiotics under commercial conditions, two to three weeks before farrowing until weaning, had a

significant decrease in the mortality rate of suckling piglets.

Fuller (1989) concluded that the growth stimulatory effect in itself is bound to be variable. It will only operate when the animals are stressed by the presence of a growth depressing micro flora. However, the same applies to all antimicrobial growth promoters including, antibiotics.

Future developments in probiotics

Probiotics are in the early stages of use. Future developments will attempt to discover more effective strains and more about their mode of action when supplemented in the diets of animals (Fuller, 1989).

On the other hand probiotics may become an important part of human diets. Matsuzaki (1997) found that the addition of *Lactobacillus casei* either by intravenous or intralesional injection, the growth and formation of both lung and axial lymph node metastases inhibit in mice. They have also found that the oral addition of *Lactobacillus casei* the recurrence of superficial bladder cancer in clinical trails with humans suppressed. According to Fuller (1989) the anti carcinogenic properties of lactobacilli fall into three categories, (a) the inhibition of tumour cells, (b) the suppression of bacteria which produce enzymes such as β- glucosidase, β- glucuronidase and azoreductase which is responsible for the release of carcinogens and (c) the destruction of carcinogens such as nitrosamines and the suppression of nitroreductase which is involved in the synthesis of nitrosamines.

The addition of lactobacillus to the diets of pigs tends to decrease the serum cholesterol levels of these animals even when additional cholesterol was supplemented in the diet. In field studies with humans the same results were obtained, but in many cases the results were variable (Fuller, 1989).

The techniques of genetic engineering will enable researchers to introduce the probiotic effect into an organism, which permanently colonizes the intestinal tract. It may also be possible to incorporate antigens from pathogenic bacteria into harmless intestinal micro flora such as lactic acid bacteria and capitalize on their ability to stimulate the immune system. Cellulytic enzymes could also be incorporated into lactobacilli. This will enable animals, like poultry and pigs, to utilize the fibre in their diets more effectively (Fuller, 1992b).

Conclusion

Thus as the resistance against antibiotics increases and more antibiotic growth promoters are banned for the use as growth promoting agents in pigs, more focus will be placed on probiotics as a viable replacement for antibiotics. By the administration of probiotics during periods of stress, the natural balance of the gut micro flora can be restored and the animal could return to its normal nutrition, growth and health status. At this moment more information on the mode of action of probiotics is needed, due to the inconsistency of results. With more information it may be possible to improve the strains of microorganisms by genetic manipulation. In this way it would be possible to bring together the ability to survive in the gut with the ability to produce the metabolites which are responsible for the improvement in animal performance (Tannock ,1992).

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Chapter 2 The influence of Effective Micro- organisms (EM) on the growth performance of the pig

Introduction

Probiotics are microorganisms, used as feed additives, which produce beneficial effects by promoting the equilibrium of the intestinal flora (Zani et al. 1998). The importance of maintaining an ideal intestinal flora in pigs has been recognized for many years. An imbalance of the intestinal micro flora can be created by stress factors which include both physiological factors, like parturition, weaning and the change of diets, and environmental factors like crowding, excessive heat, poor ventilation and sanitation. When this occurs, disease and poor performance may result (Pollmann 1986).

In the past, the most common method of suppressing the non-desirable microorganisms in the gut was to treat them with antimicrobial agents. Unfortunately such treatment can also depress non-pathogenic bacteria (Sissons 1989). In recent years, with the increasing concerns over drug residues in meat products and the risk of increased bacterial resistance against antibiotics encouraged commercial interest in probiotics as alternative therapy. It has been proposed that probiotics, which literally mean in favor of life, may have an opposite effect to antibiotics on the gut micro flora. The introducing of desirable live microorganisms into the digestive tract of the pig may restore the balance of the intestinal micro flora which may lead to an improvement in both the health and performance of the host animal.

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Lactobacilli and streptococci are the two most commonly used groups in the production of probiotics. The ways in which probiotics affect performance have been comprehensively studied, mainly for Lactobacillus strains, but studies relating to multiple culture are very scares. Several researchers have observed improvement in performance. Other workers have observed no significant improvement in performance for pigs fed a lactobacillus probiotic (Haper et al. 1983). According to Pollmann et al. (1980a) and Lessard & Brisson (1987) Lactobacillus therapy has been shown to stimulate growth and improve feed efficiency in pigs.

Effective microorganisms (EM) are an example of a mixed culture of beneficial microorganisms which primarily consist of lactobacillus (*L. plantarum* ATCC 8014 and *L. casei* ATCC 7469 S. lactis IFO12007), yeast (Saccharomyces cerevisiae IFO 0203, Canida utilis IFO 0619, actinomycetes (Streptomyces albus ATCC3004, Streptmuces griseus IFO 3358) and fermenting fungi (Aspergillus oryzae IFO 5770, Mucor hiemalis IFO 8567) (Phillips & Phillips 1996). However, currently there is no literature is available on the influence of EM on animal performance.

The first hypothesis of this study was, that the growth performance of the growing-finishing pig was not improved by the different dietary inclusion levels of EM and the second hypothesis that EM did improved growth performance over a commercial antibiotic

Materials and Methods

Animals and housing

Forty- five Large White x Landrace castrated boars were used to determine the effectiveness of EM on growth, when a 18% crude protein grower meal diet was fed *ad libitum*. The piglets were obtained from a commercial pig breeder at the age of four weeks and moved to the Research farm of the University of Pretoria.

The pigs were housed in groups of 3 for Week 5 and in groups of 2 during Week 6 and 7. The animals were kept on a commercial creep diet to minimize the stress associated with moving them. However during Week 6 the diet was change to a weaner diet, which they received until the end of Week 7. At the end of Week 6 the weaner diet was supplemented with EM Bokhasi and an antibiotic (Table 2.1 and Table 2.2). This marked the official start of the study. From the beginning of Week 8 until the end of the study the animals received the 18% CP grower diet (Table 2.1 and Table 2.2).

Housing

From the age of eight weeks, the pigs were individually housed in pens (2 x 3m) with two thirds concrete and one-third slat floors. Pens were cleaned regularly. Each pig remained in the pen allotted to him at the start of the study to prevent cross-contamination of microorganisms between pigs of different treatments. The pens were arranged into 5 blocks with 10 pens per block all within one house. Each pig was ear notched for identification purposes. The house was thoroughly washed and disinfected by a group of professional industrial cleaners under supervision of Rainbow Feeds Bk. This was done to make sure that existing microflora numbers inside the house were reduced to a large extent. Each pen had its own water nipple to which the pigs had access at all times during the study. One pig died during Week 5 and another one was removed from the study during Week 6. No more mortalities occurred during the rest of the test

i15796 22x
b150 9571x

period.

Experimental design

A block design with 5 experimental treatments was used to conduct this study. Due to the fact that 2 pigs had died before the start of the study, 3 of the 5 treatments had 9 pigs and the remaining 2 only 8 pigs each. The pigs were randomly allocated, according to body mass, to the 5 blocks and then randomly to each treatment within the specific block. In each of the first four blocks there were 2 pigs per treatment and only one pig per treatment in Block 5.

Treatments

The different treatment groups consisted of three levels of EM Bokhasi, a control group with no feed additives added to the diet and a treatment where a commercial antibiotic was added to the diet. The EM was prepared in a Bokhasi according to the specification of the Apnan user=s manual (Phillips & Phillips, 1996) whereby 1l EM Stocks were mixed with 5 l molasses and 100 l water to form extended EM. This extended EM was then added to a mixture of organic materials (Table 2.3). This organic mixture was then sealed in drums and allowed to ferment anaerobically for three weeks to form EM Bokhasi. This Bokhasi was then mixed into the grower ration, formulated to take into account the nutrients which may be derived from this Bokhasi but no correction was made for the nutrients that could have been derived from the EM *per se*. The first three treatments consisted of different levels of EM treatments:

EM 1% treatment with an inclusion level of 1% EM Bokhasi;

EM 2% treatment with an inclusion level of 2% EM Bokhasi and,

EM 3 % treatment with an inclusion level of 3% EM Bokhasi.

The last to treatments consisted of the:

Antibiotic treatment which contained a commercial antibiotic with inclusion levels of 40 ppm from weaning up to a body mass of 30 - 40 kg after which it was lowered to 20 ppm and,
Control treatment which contained no feed additives.

The same treatments were used for both the weaner and grower diets. The protein, dry matter ether extract and ash contents of the experimental diets were chemically analyzed according to the methods of the AOAC (1990). The Wijkströhm method (Application note of tecator AN 02/78) was used to determine the crude fibre content of the diets. Feed and Bokhasi were formulated and supplied by Rainbow Feeds Bk (Table 2.4).

Parameters

Pigs were weighed on a weekly basis, without prior removing of feed and water. Feed intake was measured weekly throughout the experimental period. Feed was supplied in self feeders, which was check daily. On Fridays the self feeders were emptied and the amount of feed left was then subtracted from the amount that was offered to the pigs. From these data the daily feed intake, average daily gain and feed conversion ratios were calculated. The backfat thickness ($T_{2/3}$) was determined with a Reco- backfat thickness meter, during the last two weeks of the study, at a position 5 cm dorsal, between the second and third last ribs. The measurements for the two weeks were then corrected to a body mass of 86 kg.

Statistical analysis

Data were analyzed using the SAS® system. This system is an integrated system of software providing complete control over data management, analysis, and presentation and is marketed by SAS Institute South Africa (Pty) Ltd, 1st Floor North Wing, President Place, 1 Hood Avenue, Rosebank, P. O. Box 3469, Parklands 2121, South Africa. No other reference or individual product identification or procedure identification is required and no reference to year or version number or release number etc. is required. This was as requested by the Institute. Data were subjected to analysis of variance using the General Linear Models (GLM) procedure, with each pig considered an experimental unit. Due to the experimental design it was possible to analyze treatment and block effects, but for the purpose of this study only treatment effects were investigated. Regression equations were determined by the PROC REG procedures of SAS. Values reported herein are least square means.

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Table 2.1 Physical composition of the basal experimental diet.

Item	Pig Weaner[a]	Pig Grower[a]
	% (Dry basis)	% (Dry basis)
Yellow Maize	49.70	52.20
Wheat Bran	16.30	14.30
Sunflower oilcake	10.00	15.00
Full Fat Soya	20.00	15.00
Fish meal	1.20	-
Monocalcium Phosphate	0.51	0.63
Limestone	1.30	1.85
Salt	0.39	0.40
Lysine HCl	0.24	0.24
Pig grower*	0.50	0.50

[a] Different treatments consisted of 1, 2, 3 % EM Bokhasi , 20 ppm Antibiotic and a control.

* Premix (Pig grower) supplied the following per ton; Vitamin A, 6 000 000 IU; Vitamin D, 1 200 000 IU; Vitamin E, 20 000 IU; Thaimin, 1 000 mg; Rovimix B2, 3 000 mg; Vitamin B12, 20 mg; Vitamin K3, 1 000 mg; Niacin, 20 000 mg; Pantothenic Acid, 8 000 mg; Biotin, 20 mg; Choline, 250 g; Anti oxidant, 40 g; Iron, 60 g; Manganese, 40 g; Copper, 125 g; Zinc, 100 g; Magnesium, 100 g; Iodine, 1 g; Selenium, 150 mg; Carrier, 3 kg.

Table 2.2 Calculated analysis of basal experimental diet.

Nutrient	**Pig weaner**	**Pig growth**
	% (Dry basis)	**% (Dry basis)**
Crude protein	18.84	18.00
Lysine	1.06	0.95
Methionine	0.34	0.33
Methionine + Cystine	0.70	0.70
Isoleucine	0.79	0.75
Tryptophan	0.23	0.22
Threonine	0.68	0.64
Crude Fibre	5.23	5.48
Calcium	0.79	1.00
Total Phosphorus	0.59	0.60
Sodium	0.19	0.18
DE Swine	13.80 MJ/kg	13.50 MJ/kg

* Different treatments contained the following, 1 % EM Bokhasi, 2 % EM Bokhasi, 3 % EM Bokhasi and 20 ppm of a commercial antibiotic.

* Diets containing EM Bokhasi were formulated to take into account the nutrients which may be derived from this Bokhasi but no correction was being made for the nutrients that could have derived from the EM *per se*.

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Table 2.3 Composition of EM Bokhasi

Item	% (Dry basis)
Hominy Chop	15.00
Wheat Bran	70.00
Full fat Soya	5.00
Bone meal	5.00
Fish meal	5.00
Nutrients	**% (Dry basis)**
Crude Protein	17.65
Lysine	0.85
Methionine	0.29
Fat	0.88
Crude Fibre	8.85
Calcium	1.36
Phosphorus	1.29

*Diets containing EM Bokhasi were formulate to take into account the nutrients which may be derived from this Bokhasi but no correction was being made for the nutrients that could have derived from the EM *per se*.

Table 2.4 Chemical analysis of treatments on a dry basis *.

Nutrient	EM 1 %	EM 2 %	EM 3 %	Antibiotic	Control
	%	%	%	%	%
Dry Matter	90.477	90.656	90.000	90.748	90.386
Crude Protein	20.144	18.787	19.858	19.450	19.675
Crude Fibre	9.909	10.173	10.217	10.707	9.728
Ether extract	7.698	7.192	7.850	7.322	7.470
Ash	6.922	7.252	7.013	7.119	6.825

* Diets containing EM Bokhasi were formulate to take into account the nutrients which may be derived from this Bokhasi but no correction was being made for the nutrients that could have derived from the EM *per se*.

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Results

- Overall Results

The overall effect of the influence of different Levels of EM Bokhasi and a commercial antibiotic on the growth performance of the pig are shown in Table 2.5. The three different levels of EM Bokhasi and a commercial antibiotic did not improve average daily gain over the 12 week growth period neither mean body weight gain nor the feed conversion ratio ($P>0.05$). However, the 2 % and 3 % bokhasi inclusion levels had a significantly ($P<0.05$) lower mean feed intake than the commercial antibiotic and during weeks one to three the antibiotic did have a higher average daily gain than the EM 2% and EM 3% treatments.

Details of the regression equations used to describe the response for the dietary EM and antibiotic treatments are given in Table 2.6. Predicted body mass values for the treatments against time are shown in Figure 2.1. It is evident from the regression equation that the antibiotic had a higher growth coefficient than all the other treatments. This may be directly associated with the higher growth rate of the antibiotic treatment during weeks 1 to 3.

The effect of the addition of EM and a commercial antibiotic to the diets of growing pigs on the $T_{2/3}$ back fat thickness are shown in Table 2.7. No significant results between different treatments were recorded ($P>0.05$).

- Cumulative weekly results

Table 2.8 shows the cumulative weekly results obtained for weekly cumulative body mass gain, feed intake and feed conversion ratio after the addition of different levels of EM Bokhasi and a

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commercial antibiotic to the diets of growing pigs. During week 3 and 4 the antibiotic had a higher cumulative body mass (P<0.05) than the EM 3 % treatment.

Significant differences (P<0.05) were recorded for the cumulative feed intake between the different dietary treatments. During weeks 2, 3, 4, 5 and 8 the antibiotic had a significantly higher feed intake than the EM 1 % treatment and a significantly higher feed intake than the EM 2 and EM 3 % treatments over the whole experimental period. A higher feed intake (P<0.05) was also recorded for the antibiotic vs. the control treatment during weeks 2 and 3.

Significant results for the cumulative feed conversion ratio were only recorded during weeks 7 and 10 between the antibiotic and EM 3 % treatments. The antibiotic treatment had a higher feed conversion (P<0.05) than the EM 3 % treatment during these two periods.

- Weekly performance

The influence of the addition of different levels of EM Bokhasi and a commercial antibiotic are shown in Table 2.9. Significant results between the different treatments were recorded for the average weekly gain, average daily gain, average daily feed intake and feed conversion ratio within different weeks. The antibiotic had higher average weekly and average daily gains (P<0.05) than; the EM 2 %, EM 3 % and control treatments during week 2, the EM 3 % treatment during week 8 and the EM 1 % treatment during week 11. The control treatment had significantly higher gains than the EM 1 %, EM 2 % and EM 3 % treatments during week 9.

The EM 1 treatment had a significantly (P<0.05) lower feed intake than the antibiotic during weeks 2 and 5 and the control a significantly lower intake than the antibiotic during week 2.

Significant results ($P<0.05$) were recorded between the antibiotic and the EM 2 % and EM 3 % treatments during weeks 2, 3, 5, 7 and 8. The antibiotic had a higher feed intake ($P<0.05$) than both the EM 2 % and EM 3 % treatments. The EM 2 % treatment had also a significantly lower feed intake than the antibiotic during weeks 4 and 10.

During week 7 both the antibiotic and EM 1 treatment had a higher ($P<0.05$) feed conversion ratio than the EM 3 % treatment and the EM 2 treatment a higher feed conversion ($P<0.05$)than the control treatment. No other significant ($P<0.05$) results were recorded.

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Table 2.5 LSMEANS and type III P- value of the mean performance of the growing pig *

Growth performance						
Item	**EM 1 %**	**EM 2 %**	**EM 3 %**	**Antibiotic**	**Control**	**P- value**
Average daily gain, kg						
Weeks 1 to 3	0.7119	0.6613[a]	0.6695[b]	0.7833[ab]	0.7066	0.1451
SEM	0.0343	0.0380	0.0343	0.0380	0.0343	
Weeks 3 to 6	0.9520	0.9426	0.9308	0.9813	0.9837	0.7855
SEM	0.0362	0.0401	0.0362	0.0401	0.0362	
Weeks 1 to 6	0.8319	0.8019	0.8002	0.8823	0.8451	0.3238
SEM	0.0299	0.0331	0.0299	0.0331	0.0299	
Weeks 6 to 9	0.8447	0.8440	0.8208	0.8886	0.9082	0.5829
SEM	0.0423	0.0468	0.0423	0.0468	0.0423	
Weeks 1 to 9	0.8362	0.8160	0.8071	0.8844	0.8661	0.3532
SEM	0.0300	0.0333	0.0300	0.0333	0.0300	
Weeks 9 to 12	0.9915	0.9988	1.1026	1.0880	1.0272	0.4130
SEM	0.0502	0.0556	0.0502	0.0556	0.0502	
Weeks 1 to 12	0.8644	0.8492	0.8608	0.9214	0.8954	0.3222
SEM	0.0259	0.0287	0.0259	0.0287	0.0259	
Mean body weight gain, kg						
Mean weeks 1 to 12	44.2817	43.5298	42.4438	46.4986	44.4993	0.4828
SEM	1.5408	1.7069	1.5408	1.7069	1.5408	
Mean feed intake, kg						
Mean weeks 2 to 11	16.0129	15.2665[a]	15.6440[b]	17.4978[ab]	16.5140	0.1187
SEM	0.5912	0.6550	0.5912	0.6550	0.5912	

Growth performance						
Item	**EM 1 %**	**EM 2 %**	**EM 3 %**	**Antibiotic**	**Control**	**P- value**
Feed conversion ratio						
Weeks 1 to 12	2.5891	2.5155	2.5294	2.6603	2.5932	0.5738
SEM	0.0651	0.0722	0.0651	0.0722	0.0651	

*LSMEANS in the same row and column bearing same superscripts differ significantly ($p<0.05$)

Table 2.6 Regression relationship between cumulative body weight (Y) and time (X)

Regression relationship		
Treatment	**R^2**	**Regression equations**
EM 1 %	0.9731	$y = 3.9814 + 6.2329x$
EM 2 %	0.9387	$y = 4.1240 + 6.1363x$
EM 3 %	0.9615	$y = 2.5740 + 6.1666x$
Antibiotic	0.9471	$y = 4.1013 + 6.5965x$
Control	0.8711	$y = 3.0437 + 6.4106x$

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Table 2.6 Regression relationship between cumulative body weight (Y) and time (X)

Regression relationship		
Treatment	**R^2**	**Regression equations**
EM 1 %	0.9731	y = 3.9814 + 6.2329x
EM 2 %	0.9387	y = 4.1240 + 6.1363x
EM 3 %	0.9615	y = 2.5740 + 6.1666x
Antibiotic	0.9471	y = 4.1013 + 6.5965x
Control	0.8711	y = 3.0437 + 6.4106x

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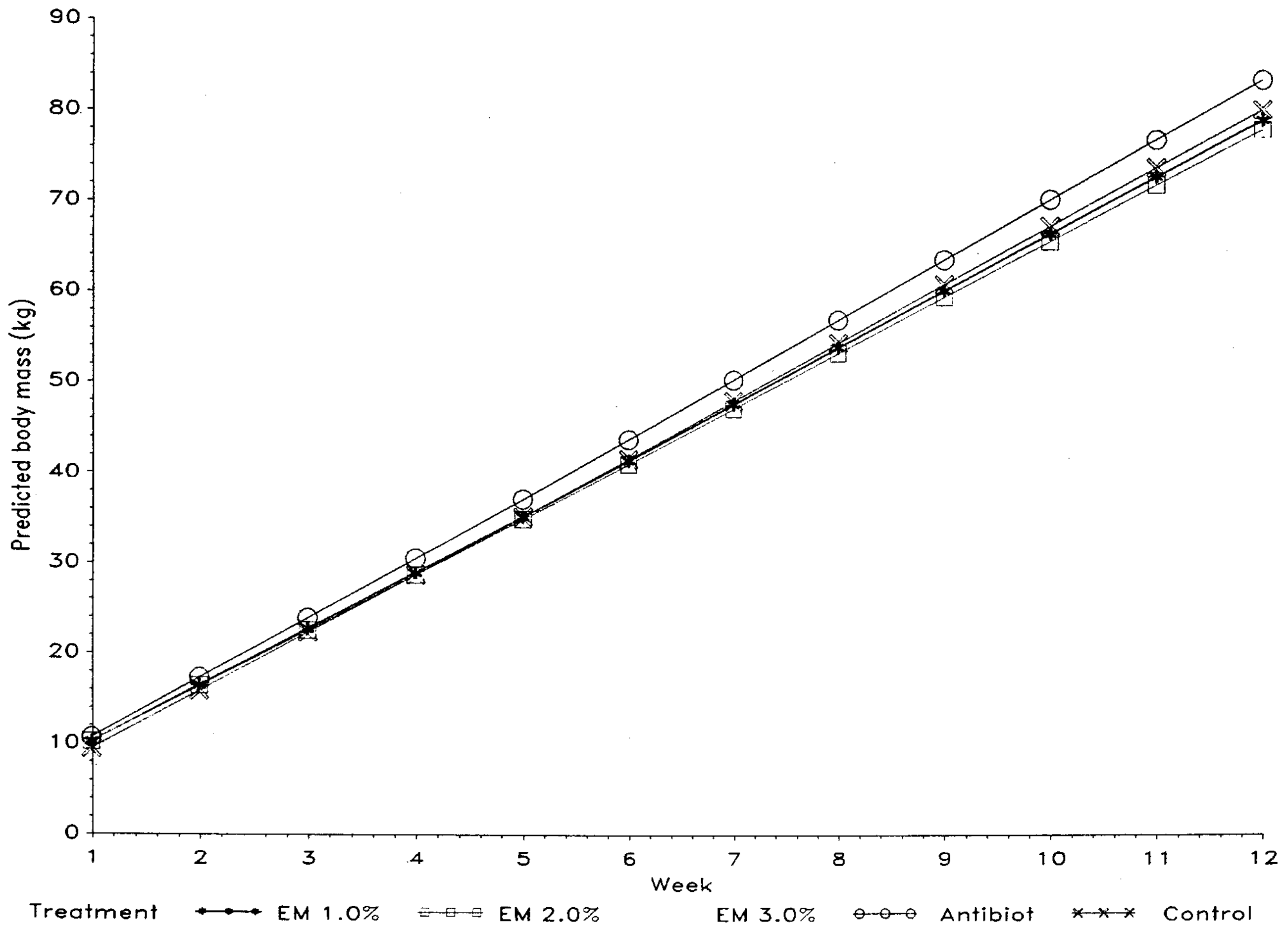

Fig 2.6 The predicted body mass for the different treatments over 12 weeks.

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Table 2.7 LSMEANS and type III P-value of the $T_{2/3}$ backfat thickness of the growing pig *

Backfat Thickness $T_{2/3}$ (mm)						
Treatment	**Week 10**	**SEM**	**Week 11**	**SEM**	**Corrected to 86 kg**	**SEM**
EM 1	11.878	0.752	11.922	0.743	11.833	1.301
EM 2	12.225	0.833	12.325	0.823	12.297	1.441
EM 3	12.767	0.752	12.256	0.743	12.688	1.301
Antibiotic	13.850	0.833	12.700	0.823	12.517	1.441
Control	12.656	0.752	12.922	0.743	12.759	1.301
P-value	0.462		0.889		0.987	

*LSMEANS in the same row and column bearing same superscripts differ significantly ($p<0.05$)

Table 2.8 LSMEANS and type III P- value of the cumulative weekly performance of the growing pig: body mass, cumulative feed intake (CFI) and cumulative feed conversion ratio(CFCR)*

Growth performance						
	Body mass kg	**SEM**	**CFI kg**	**SEM**	**CFCR**	**SEM**
Week 1						
EM 1 %	12.5518	0.7064				
EM 2 %	12.7416	0.7826				
EM 3 %	11.5518	0.7064				
Antibiotic	12.7416	0.7826				
Control	11.9962	0.7064				
P- value	0.7121					
Week 2						
EM 1 %	17.1870	0.9395	9.5918[a]	0.3819	2.0881	0.9608
EM 2 %	17.6083	1.0408	8.8191[b]	0.4231	2.3720	1.0644
EM 3 %	15.9648	0.9395	8.8807[c]	0.3819	2.1807	0.9608
Antibiotic	17.6708	1.0408	10.7566[abcd]	0.4231	1.8324	1.0644
Control	17.2981	0.9395	9.5474[d]	0.3819	4.0500	0.9608
P- value	0.7153		0.0107		0.5032	
Week 3						
EM 1 %	21.9092	1.0441	20.8462[a]	0.7795	2.0444	0.1262
EM 2 %	21.2958	1.1567	19.6541[b]	0.8635	2.1746	0.1398
EM 3 %	20.1314[a]	1.0441	19.3685[c]	0.7795	2.0508	0.1262
Antibiotic	23.2958[a]	1.1567	23.1416[abcd]	0.8635	2.0334	0.1398
Control	21.4092	1.0441	20.9351[d]	0.7795	2.2869	0.1262
P- value	0.3526		0.0192		0.5537	

	Body mass kg	SEM	CFI kg	SEM	CFCR	SEM
Week 4						
EM 1 %	27.5018	1.2433	34.6403	1.2869	2.1960	0.0780
EM 2 %	26.6291	1.3774	32.8783[a]	1.4257	2.2276	0.0864
EM 3 %	25.6129[a]	1.2433	32.9959[b]	1.2869	2.1770	0.0780
Antibiotic	29.1916[a]	1.3774	38.0658[ab]	1.4257	2.2428	0.0864
Control	26.8351	1.2433	34.7737	1.2869	2.2841	0.0780
P- value	0.3862		0.0646		0.8815	
Week 5						
EM 1 %	33.0074	1.3179	49.6459[a]	1.8814	2.1689	0.0597
EM 2 %	32.3291	1.4600	47.7033[b]	2.0842	2.1659	0.0661
EM 3 %	31.1185	1.3179	48.0459[c]	1.8814	2.1564	0.0597
Antibiotic	34.7666	1.4600	55.1408[abc]	2.0842	2.2373	0.0661
Control	32.6740	1.3179	50.5681	1.8814	2.1867	0.0597
P- value	0.4477		0.0759		0.8952	
Week 6						
EM 1 %	40.0962	1.5630	66.6625	2.4647	2.2015	0.0516
EM 2 %	39.5916	1.7315	64.1908[a]	2.7305	2.2194	0.0572
EM 3 %	38.2629	1.5630	64.3181[b]	2.4647	2.2040	0.0516
Antibiotic	42.5291	1.7315	73.5408[ab]	2.7305	2.2986	0.0572
Control	40.5407	1.5630	67.9292	2.4647	2.2534	0.0516
P- value	0.4541		0.0874		0.6710	

	Body mass kg	SEM	CFI kg	SEM	CFCR	SEM
Week 7						
EM 1 %	47.4944	1.7470	84.3322	3.0986	2.2582	0.0506
EM 2 %	46.4250	1.9354	81.0016[a]	3.4327	2.2506	0.0560
EM 3 %	45.1611	1.7470	81.5430[b]	3.0986	2.2146[a]	0.0506
Antibiotic	49.8000	1.9354	92.9891[ab]	3.4327	2.3702[a]	0.0560
Control	47.4944	1.7470	85.9229	3.0986	2.2958	0.0506
P- value	0.4716		0.0853		0.2969	
Week 8						
EM 1 %	54.4981	1.9466	102.3629[a]	3.8356	2.4355	0.0555
EM 2 %	53.4958	2.1565	98.6916[b]	4.2492	2.4159	0.6157
EM 3 %	52.7759	1.9466	99.3296[c]	3.8356	2.4308	0.0555
Antibiotic	57.0583	2.1565	113.2166[abc]	4.2492	2.5522	0.0615
Control	54.7759	1.9466	104.8074	3.8356	2.5292	0.0555
P- value	0.6269		0.0940		0.3163	
Week 9						
EM 1 %	59.2296	1.9889	120.6592	4.5983	2.5140	0.0523
EM 2 %	58.3916	2.2033	115.5208[a]	5.0941	2.4755	0.0580
EM 3 %	56.8407	1.9889	117.0925[b]	4.5983	2.5314	0.0523
Antibiotic	62.2666	2.2033	132.6208[ab]	5.0941	2.6257	0.0580
Control	58.7296	1.9889	123.5481	4.5983	2.5163	0.0523
P- value	0.4563		0.1163		0.3989	

Table 2.9 LSMEANS and type III P- value of the weekly performance of the growing pig: average weekly gain (AWG), average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR)*

Growth Performance^{X}								
	AWG kg/w	**SEM**	**ADG kg/d**	**SEM**	**ADFI kg/d**	**SEM**	**FCR g/kg**	**SEM**
Week 1								
EM 1	4.635	0.483	0.662	0.068				
EM 2	4.867	0.535	0.695	0.076				
EM 3	4.413	0.483	0.630	0.068				
Antibiotic	4.929	0.535	0.704	0.076				
Control	5.301	0.483	0.757	0.068				
P-value	0.744		0.744					
Week 2								
EM 1	4.723	0.422	0.675	0.060	1.370^{a}	0.055	2.088	0.961
EM 2	3.688^{a}	0.467	0.527^{a}	0.067	1.259^{b}	0.060	2.372	1.064
EM 3	4.167^{b}	0.422	0.595^{b}	0.060	1.269^{c}	0.055	2.180	0.961
Antibiotic	5.625^{abc}	0.467	0.804^{abc}	0.067	1.537^{abcd}	0.060	1.832	1.064
Control	4.111^{c}	0.422	0.587^{c}	0.060	1.364^{d}	0.055	4.050	0.961
P-value	0.036		0.036		0.011		0.503	
Week 3								
EM 1	5.593	0.361	0.798	0.052	1.608	0.064	2.029	0.091
EM 2	5.334	0.400	0.762	0.057	1.548^{a}	0.071	2.051	0.101
EM 3	5.481	0.361	0.783	0.052	1.498^{b}	0.064	1.973	0.091
Antibiotic	5.896	0.400	0.842	0.057	1.769^{ab}	0.071	2.129	0.101
Control	5.426	0.361	0.775	0.052	1.627	0.064	2.133	0.091
P-value	0.854		0.854		0.071		0.690	

	AWG kg/w	SEM	ADG kg/d	SEM	ADFI kg/d	SEM	FCR g/kg	SEM
Week 4								
EM 1	5.056	0.368	0.787	0.053	1.971	0.080	2.640	0.224
EM 2	5.700	0.408	0.814	0.058	1.889[a]	0.089	2.327	0.248
EM 3	5.056	0.368	0.787	0.053	1.947	0.080	2.652	0.224
Antibiotic	5.575	0.408	0.796	0.058	2.132[a]	0.089	2.734	0.248
Control	5.839	0.368	0.834	0.053	1.977	0.080	2.397	0.224
P-value	0.960		0.960		0.347		0.667	
Week 5								
EM 1	7.089	0.471	1.013	0.067	2.144[a]	0.092	2.146	0.127
EM 2	7.263	0.521	1.038	0.074	2.118[b]	0.102	2.136	0.141
EM 3	7.144	0.471	1.021	0.067	2.150[c]	0.092	2.137	0.127
Antibiotic	7.763	0.521	1.109	0.074	2.439[abc]	0.102	2.290	0.141
Control	7.867	0.471	1.124	0.067	2.256	0.092	2.143	0.127
P-value	0.671		0.671		0.129		0.905	
Week 6								
EM 1	7.398	0.450	1.057	0.064	2.431	0.096	2.489	0.297
EM 2	6.833	0.499	0.976	0.071	2.355[a]	0.105	2.745	0.329
EM 3	6.898	0.450	0.985	0.064	2.325[b]	0.096	2.535	0.297
Antibiotic	7.271	0.499	1.039	0.071	2.629[ab]	0.106	2.910	0.329
Control	6.954	0.450	0.993	0.064	2.480	0.096	3.163	0.297
P-value	0.877		0.877		0.231		0.481	

	AWG kg/w	SEM	ADG kg/d	SEM	ADFI kg/d	SEM	FCR g/kg	SEM
Week 10								
EM 1	8.324	0.693	1.189	0.099	2.770	0.122	2.862	0.390
EM 2	7.417	0.767	1.060	0.110	2.533[a]	0.135	2.528	0.432
EM 3	9.269	0.693	1.324	0.099	2.727	0.122	2.148	0.039
Antibiotic	8.042	0.767	1.149	0.110	2.971[a]	0.135	2.699	0.432
Control	8.157	0.693	1.165	0.099	2.834	0.122	2.631	0.390
P-value	0.479		0.479		0.202		0.751	
Week 11								
EM 1	5.557[a]	0.573	0.794[a]	0.082	2.868	0.120	4.160	0.881
EM 2	6.567	0.635	0.938	0.091	2.773	0.133	2.906	0.976
EM 3	6.169	0.573	0.881	0.082	2.894	0.120	3.437	0.881
Antibiotic	7.192[a]	0.635	1.027[a]	0.091	3.080	0.133	2.929	0.976
Control	6.224	0.573	0.889	0.082	3.108	0.120	4.778	0.881
P-value	0.411		0.411		0.264		0.526	

* LSMEANS in the same row and column bearing same superscripts differ significantly ($p<0.05$)

[x] Calculated for each individual week of the trail period

Discussion

The inclusion of EM and a commercial antibiotic did not show any beneficial effect on the overall body weight gain, feed conversion ratio or backfat thickness. However, a higher feed intake for the antibiotic treatment in comparison with the EM 2% and EM 3% treatments were recorded. Although not significant, the antibiotic treatment tended to have a higher mean body weight gain than these two treatments. This may be the reason why there was no improvement in the feed conversion ratio for the EM 2% and EM 3% treatment over the antibiotic treatment.

During the first three weeks of this study the antibiotic had significantly improved the feed intake in comparison with the other treatments. The reason for this may be that the antibiotic might have reduced the digestive disorders associated with weaning and the change in the diet, in the pigs receiving this treatment. This may also be evident for the average daily gain during this period although not significant, except for the EM 2% and EM 3% treatments.

The results for the first three weeks of this study are in accordance with the results of Pollmann et al. (1980a) and Collington et al. (1990) who found that the addition of either a probiotic or an antibiotic regardless of the source, improved daily gain and feed conversion in four week old pigs. In the second phase of the study of Pollmann et al. (1980a) the inclusion of a probiotics did not improve the performance of growing-finishing pigs, this is in accordance with the results of this study after the first three weeks. However, Pollmann et al. (1980a) did find a beneficial effect of the supplementation of antibiotics in the diet of growing- finishing pigs on average daily gain and feed conversion ratios. This is in contrast with the overall results of this study where the

supplementation of an antibiotic only improved feed intake.

Harper et al. (1983) and Apgar et al. (1993) observed no improvement in the performance of weaned pigs after the supplementation of an antibiotic or a lactobacillus probiotic. However, the feed intake of the pigs supplemented with the antibiotic was higher during the finishing phase than the feed intake of the pigs receiving the probiotic. According to Apgar et al. (1993) the variation in response to microbial supplementation may be attributed to a lack of viable microorganisms in probiotic preparations.

Zani et al. (1998) found a significant improvement in weight gain and feed conversion ratio when a Bacillus probiotic was supplemented to piglets just after weaning. The same researchers found that an increase in concentration or level of inclusion did improve the weight gain and feed conversion ratios. In this study no effect of inclusion level on the performance of the pigs could be detected. However, Lessard & Brisson (1987) observed an improvement in weight gain and feed intake when the diet of growing pigs were supplemented with a lactobacillus probiotic, but no improvement in feed conversion were observed. In contrast Hale & Newton (1979) did observe an improvement in feed conversion but not in weight gain when a nonviable lactobacillus product were supplemented.

No significant differences in the $T_{2/3}$ backfat thickness were recorded. This is in agreement with Apgar et al. (1993) who found no significant effect of the supplementation of a probiotic on carcass measurements. However, there was a trend for the EM 1% treatment to lower backfat thickness.

Conclusion

According to the results of this study the hypothesis that EM improve growth performance over the commercial antibiotics could be rejected during the first few weeks after weaning. However, the hypothesis that the supplementation of EM did not improve the performance of the growing pig could not be rejected, but on the other hand no negative effects of EM on the performance of the pigs could be detected in this study.

According to (Fuller 1989) probiotics will only operate as a growth stimulant when the animals are stressed by the presence of growth depressing micro flora. Probiotics increase the numbers of desirable microorganisms in the gut thereby swinging the balance towards a more favourable micro flora (Thacker 1988). In addition, this balancing effect on the gut micro flora may not necessarily be reflected by the performance of the animal (Scheuermann 1993). The very strict hygiene control and the fact that only one diet was fed from Week 2 till the end of the study may be the reason why the pigs in this study did not respond to the supplementation of probiotics. According to Visek (1978) antibiotics is also more effective as growth promoters were hygiene control is poor.

One must bear in mind that although no significant improvements were recorded, the results may still be of biological significance (Barrow 1992). Only a small but consistent weight gain, as with the antibiotic, may not be of statistical significance. However, for the producer who is paid for each kg of meat produced, it may be significant in terms of money when he produces a few hundred kg of pork meat.

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Chapter 3 The influence of Effective Micro-organisms (EM) on nutrient digestibilities of growing pigs

Introduction

Probiotics are defined as the addition of a live microbial supplement, which beneficially affects the host by improving its intestinal microbial balance (Fuller 1989). Nearly all the literature available speculates about the mode of action of probiotics and how it influences the growth performance of the host animal, but the literature does not indicate whether this growth response stem directly from improved digestive performance or indirectly from the suppression of gut pathogens on the gastrointestinal function or metabolism (Sissons1989). The reports of the influence of probiotic supplementation on nutrient digestibility and N retention are limited and the findings inconsistent (Kornegay et al. 1996).

According to Kornegay et al. (1996) some researchers found significant improvements in the digestion of dry matter, crude fibre and both N digestion and retention. However, Hale & Newton (1979) did not found an improvement in the retention and digestibility of N, or the digestibility of dry matter, ether extract or nitrogen-free extract when a nonviable lactobacillus product was included in the diet of growing pigs.

Effective microorganisms (EM) are an example of a mixed culture of beneficial microorganisms which primarily consist of lactobacillus, yeast, actinomycetes and fermenting fungi (Phillips & Phillips, 1996). However, currently there is no literature available on the influence of EM on the

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digestive performance of the growing pig.

Due to the limited reports of the influence of probiotics on the digestive performance of the pig, the hypothesis tested in this study was that nutrient digestibility and N retention of a growing pig diet was not improved by different dietary levels of EM neither by a commercial antibiotic.

Materials and Methods

Animals

Thirty Large White x Landrace castrated boars with an average body weight of approximately 55 kg were used in a conventional digestibility experiment to investigate the influence on nutrient digestibility and N retention of adding three levels of EM and a commercial antibiotic to a 18 % grower meal diet (Table 3.1 & 3.2).

Housing

The pigs were obtained from a commercial pig breeder at the age of four weeks and moved to the Research farm of the University of Pretoria. They were housed in-groups of two until they have reached an age of eight weeks where after, they were individually housed. Each pig were ear notched for identification purposes and kept in the same pen during the growth period to prevent cross contamination of micro-organisms between pigs of different treatments. Each pen had its own water nipple to which the pigs had access at all times. From week eight the pigs received a grower diet *ad libitum*, which were supplemented with EM Bokhasi and an antibiotic.

Treatments

The different treatment groups consisted of three levels of EM Bokhasi, a control group with no feed additives added to the diet and a treatment where a commercial antibiotic was added to the diet. The EM was prepared in a Bokhasi according to the specification of the Apnan user=s manual (Phillips & Phillips 1996) whereby 1l EM Stocks were mixed with 5 l molasses and 100 l water to form extended EM. This extended EM was then added to a mixture of organic materials (Table 3.3). This organic mixture was then sealed in drums and allowed to ferment anaerobically for three weeks to form EM Bokhasi. This Bokhasi was then mixed into the grower ration, formulated to take into account the nutrients which may be derived from this Bokhasi but no correction was being made for the nutrients that could have been derived from the EM *per se*.

The first three treatments consisted of different levels of EM,

EM 1% treatment with an inclusion level of 1% EM Bokhasi,

EM 2% treatment with an inclusion level of 2% EM Bokhasi and,

EM 3% treatment with an inclusion level of 3% EM Bokhasi.

The last two treatments consisted of the:

Antibiotic treatment which contained a commercial antibiotic with inclusion levels of 20 ppm and,

Control treatment which contained no feed additives.

The protein, dry matter ether extract and ash contents of the experimental diets were chemically analyzed according to the methods of the AOAC (1990) and the Wijkströhm method (Application note of tecator AN 02/78) for determining the crude fibre content of the diets. Feed and Bokhasi were formulated and supplied by Rainbow Feeds Bk. (Table 3.4).

Experimental design

Only ten metabolic crates were available at all times therefore, the experimental design had to be

over three time periods. Ten pigs were used in each of the three time periods. The ten pigs were randomly allocated to the five different treatments within each of the three time periods. In each of the three time periods only two pigs per treatment were used, but over the entire study a total of six pigs per treatment was used. A carry over design was not used due to the fact that no literature on the establishment of EM in the gut after administration was available.

Experimental procedure

At the onset of the digestibility experiment the pigs were approximately 14 weeks old. Due to the fact that the pigs were raised on the experimental diets, no adaptation periods for the pigs on the experimental diets were necessary. On the other hand the pigs were given some time to adapt to the metabolic crates. Feed intake was used as the parameter to determine when the pigs had fully adapted to the crates. Before the pigs were moved to the crates average voluntary daily feed intake for the ten pigs was determine and as soon as the pigs consumed all the feed offered in the crate, the collection period started.

On Day 1 the pigs were given a fixed amount of feed at approximately 09h00 and for the following four days the same amount of feed was given at the same time each morning after the feces and urine had been collected. Water was supplied as soon as the pigs consumed all their feed and again in the afternoon. The metabolic crates, feeders and urine pans were cleaned regularly during each collection period. All apparatus were also thoroughly cleaned before and after each collection period (Schneider & Flatt 1975). Metabolic crates were all located in one house, which were properly cleaned and disinfected before the onset of the study by a group of professional industrial cleaners under supervision of Rainbow Feeds Bk.

The following procedure was carried out each morning:

- The total amount of feces voided was collected, weighed and mixed. A random sample was taken and stored at -15 ^{0} C.
- The total volume of urine passed was measured and a 10 % sample was stored at 15 ^{0} C. To minimize nitrogen loss, approximately 10 ml Hydrochloric acid (35 %) was added to the urine containers each morning after the urine was collected.

The following was done at the end of each collection period:

-The feces collected from each pig was thoroughly mixed and a random sample off approximately 60 g was dried at 100 ^{0} C for 48 hour to determine the percentage dry matter voided by each pig. A random sample of the rest of the feces was taken for chemical analysis. The feces was dried at 65 ^{0} C for 48 hours, mixed, ground and stored in airtight containers.

-After mixing the cumulative amount of urine collected a sample of approximately 100 ml were taken, filtered and stored at 4 ^{0} C for analysis. Where necessary pH adjustments were done at the end of each collection period.

Chemical analysis

The AOAC (1990) procedures were used for the determination of dry matter, ash, N, and ether extract components of the feces and the Wijkströhm method (Application note of tecator AN 02/78) for determining the crude fibre content of the feces. The digestibility coefficients were calculated from the data obtained. The energy content of the feed, feces and urine were determined with an automatic calorific processor (CP 500). A feed and feces sample of approximately 0.5g pressed into a pellet and a 3,0ml urine sample was used for energy determinations. The urine energy determination was done with towel paper with a known energy

value, pressed into a pellet. The urine sample was then placed on this paper pellets and dried in an oven at 60 ^{0} C after which the energy determination was done (CP 500).

Statistical analysis

Data were analyzed using the SAS® system. This system is an integrated system of software providing complete control over data management, analysis, and presentation and is marketed by SAS Institute South Africa (Pty) Ltd, 1st Floor North Wing, President Place, 1 Hood Avenue, Rosebank, P. O. Box 3469, Parklands 2121, South Africa. No other reference or individual product identification or procedure identification is required and no reference to year or version number or release number etc. is required. This was as requested by the Institute. Data were subjected to analysis of variance using the General Linear Models (GLM) procedure, with each pig considered an experimental unit. Due to the experimental design it was possible to analyze treatment, time period and treatment time period interactions, but for the purpose of this study only treatment effects were investigated. Values reported herein are least square means.

Table 3.1 Composition of the basal experimental diet

Item	Pig Weaner[a]
	% (Dry basis)
Yellow Maize	49.70
Wheat Bran	16.30
Sunflower oilcake	10.00
Full Fat Soya	20.00
Fish meal	1.20
Monocalcium Phosphate	0.51
Limestone	1.30
Salt	0.39
Lysine HCl	0.24
Pig grower*	0.50

[a] Different treatments consisted of 1, 2, 3 % EM Bokhasi , 20 ppm Antibiotic and a control.

* Premix (Pig grower) supplied the following per ton; Vitamin A, 6 000 000 IU; Vitamin D, 1 200 000 IU; Vitamin E, 20 000 IU; Thaimin, 1 000 mg; Rovimix B2, 3 000 mg; Vitamin B12, 20 mg; Vitamin K3, 1 000 mg; Niacin, 20 000 mg; Pantothenic Acid, 8 000 mg; Biotin, 20 mg; Choline, 250 g; Anti oxidant, 40 g; Iron, 60 g; Manganese, 40 g; Copper, 125 g; Zinc, 100 g; Magnesium, 100 g; Iodine, 1 g; Selenium, 150 mg; Carrier, 3 kg.

Table 3.2 Calculated analysis of experimental diets

Nutrient	**Pig weaner**
	% (Dry basis)
Crude protein	18.84
Lysine	1.06
Methionine	0.34
Methionine + Cystine	0.70
Isoleucine	0.79
Tryptophan	0.23
Threonine	0.68
Crude Fibre	5.23
Calcium	0.79
Total Phosphorus	0.59
Sodium	0.19
DE Swine	13.80 MJ/kg

* Different treatments contained the following, 1 % EM Bokhasi, 2 % EM Bokhasi, 3 % EM Bokhasi and 20 ppm of a commercial antibiotic.

* Diets containing EM Bokhasi were formulated to take into account the nutrients which may be derived from this Bokhasi but no correction was being made for the nutrients that could have derived from the EM *per se*.

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Table 3.3 Composition of EM Bokhasi

Item	**% (Dry basis)**
Hominy Chop	15.00
Wheat Bran	70.00
Full fat Soya	5.00
Bone meal	5.00
Fish meal	5.00
Nutrients	**% (Dry basis)**
Crude Protein	17.65
Lysine	0.85
Methionine	0.29
Fat	0.88
Crude Fibre	8.85
Calcium	1.36
Phosphorus	1.29

*Diets containing EM Bokhasi were formulate to take into account the nutrients which may be derived from this Bokhasi but no correction was being made for the nutrients that could have derived from the EM *per se*.

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Table 3.4 Chemical analysis of pig growth diets on a dry basis*.

Nutrient	EM 1 %	EM 2 %	EM 3 %	Antibiotic	Control
	%	%	%	%	%
Dry Matter	89.116	89.235	89.417	89.6251	89.5083
Crude Protein	16.976	16.756	17.018	16.584	16.3589
Crude Fibre	9.696	7.917	8.555	9.404	9.812
Ether extract	9.420	8.818	8.722	9.013	8.937
Ash	5.303	5.755	6.501	5.649	5.914

* Diets containing EM Bokhasi were formulate to take into account the nutrients which may be derived from this Bokhasi but no correction was being made for the nutrients that could have derived from the EM *per se*.

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Results

The influence of the dietary addition of three different levels of EM Bokhasi and a commercial antibiotic on the digestibility of dry matter, nitrogen, energy, crude fibre, ether extract and ash are shown in Table 3.5.

The EM 2% treatment had a significantly ($P<0.05$) higher dry matter digestibility coefficient than the EM 3% treatment. The EM 3% treatment also had a lower dry matter digestibility coefficient than the antibiotic. No other significant results were obtained for the dry matter digestibility coefficient between the different treatments.

No significant ($p>0.05$) results were recorded between different treatments for the apparent digestible energy MJ/kg, however the EM 2% treatment had a significantly higher apparent digestible energy % than the EM 3% treatment. The ME MJ /kg value of the EM 2% treatment were also higher ($P<0.05$) than the value for both the EM 3% and control treatments. The ME/DE % of the EM 2% was also higher than the value for the control treatment. The control treatment had a significantly lower N digestibility coefficient than the EM 1%, EM 2% and antibiotic treatments but, the EM 3% treatment had again a lower N digestibility coefficient than the antibiotic treatment. The EM 2% treatment had a significantly ($P<0.05$) higher N balance as % of N intake than the control treatment.

The antibiotic had a higher ($P<0.05$) crude fibre digestibility coefficient than both the EM 2% and EM 3% treatments. On the other hand the EM 1%, EM 2% and antibiotic

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treatments had significantly ($P<0.05$) higher ether extract digestibility coefficients than both the EM 3% and control treatments.

The EM 1% treatment had a significant ($P<0.05$) lower ash absorbed % than the EM 2%, antibiotic and control treatments.

Table 3.5 LSMEANS and type III P-value of the nutrient digestibilities as influenced by the different treatments *

Nutrient Digestibilities							
Item	**EM 1**	**EM 2**	**EM 3**	**Antibiotic**	**Control**	**P-value**	**SEM**
Dry matter							
Intake, g	1254.45[a]	1299.79[b]	1273.02[c]	1451.70[abcd]	1189.64[d]	0.0276	50.58
Fecal, g	316.91	309.32[a]	352.42	367.08[ac]	309.87[c]	0.1319	18.51
Digested, g	937.54[a]	990.46[c]	920.608[b]	1084.62[abd]	879.76[cd]	0.0163	38.07
Digestibility coefficient, %	74.786	76.356[a]	72.851[ab]	75.893[b]	74.221	0.1177	0.937
Energy							
GE intake, g	24.063[a]	24.086[b]	24.483[c]	27.653[abcd]	22.701[d]	0.0323	0.967
Fecal energy, MJ/kg	5.806	5.687[a]	6.439	6.697[a]	5.801	0.1766	0.334
Urine energy, MJ/kg	0.804	0.747	0.786	0.845	0.828	0.8113	0.060
Apparent digestible energy MJ/kg	14.601	14.767	14.271	14.647	14.260	0.2046	0.177
Apparent digestible energy, %	76.008	77.199[a]	74.189[a]	76.748	74.662	0.1487	0.924
ME, MJ/kg	14.007	14.230[ab]	13.666[b]	14.072	13.585[a]	0.1347	0.191
ME/DE, %	95.924	96.347[a]	95.749	96.050	95.285[a]	0.2907	0.336

Item	EM 1	EM 2	EM 3	Antibiotic	Control	P-value	SEM
Nitrogen							
Intake, g	36.970^{ae}	36.847^{b}	37.333^{c}	42.027^{abcd}	34.363^{de}	0.0189	1.373
Fecal, g	6.915	6.918	7.780	7.487	7.341	0.4886	0.395
Digestibility coefficient, %	80.768^{a}	81.020^{b}	79.348^{c}	81.764^{cd}	77.716^{abd}	0.0053	0.670
Urine	16.668	15.869	16.839	18.308	15.075	0.3872	1.145
N balance, g	13.387	14.060	12.714	16.232^{a}	11.946^{a}	0.2673	1.359
N balance as % of N intake	37.227	40.433^{a}	33.577	36.164	30.812^{a}	0.2642	3.025
Crude fibre							
Intake , g	122.849^{a}	116.794^{b}	118.676^{c}	146.482^{abcd}	116.218^{d}	0.0030	4.974
Fecal, g	76.169	76.626	82.085	89.694^{a}	74.111^{a}	0.2419	5.081
Digested, g	46.680	40.168^{a}	36.592^{b}	56.788^{abc}	42.106^{c}	0.0129	3.640
Digestibility coefficient, %	38.633	32.008^{a}	30.650^{b}	41.144^{ab}	37.626	0.0794	2.791
Ether extract							
Intake, g	108.338	104.612^{b}	105.917	117.922^{ab}	97.407^{a}	0.0645	4.435
Fecal, g	13.018^{ab}	14.402^{c}	17.810^{ac}	15.586	16.701^{b}	0.0553	1.095
Digested, g	95.320^{a}	90.210^{b}	88.107^{c}	102.336^{bce}	80.706^{ae}	0.0239	4.120
Digestibility coefficient, %	87.759^{ab}	86.242^{df}	83.159^{acf}	86.256^{ec}	81.767^{bde}	0.0071	1.074

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Item	EM 1	EM 2	EM 3	Antibiotic	Control	P-value	SEM
Ash							
Intake, g	75.777[ab]	84.031[d]	85.776[ae]	93.255[bcd]	75.900[ce]	0.0053	3.074
Fecal, g	47.341	42.473	51.081	48.473	41.217	0.2629	3.435
Absorbed, g	28.436[ab]	41.558[a]	34.695[c]	44.782[bcd]	34.682[d]	0.0064	2.746
Absorbed, %	34.255[abc]	47.753[a]	40.222	46.591[b]	44.213[c]	0.0531	3.171

*LSMEANS in the same row and column bearing same superscripts differ ($p<0.05$)

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Discussion

Due to the fact that the pigs were raised on the experimental diets and the feed intake during the collection period were determined by the voluntary feed intake of the animals before the onset of the study, the antibiotic treatment had a significantly higher dry matter, gross energy, nitrogen and crude fibre intake. This is in correlation with the significantly higher ($P<0.05$) feed intake of the antibiotic treatment over the EM 2% and EM 3% treatments in the growth study, (Chapter 2) without any significant improvements in overall body weight nor feed efficiency. These results are in agreement with the results obtained by Harper (1983) who found higher ($P<0.05$) feed intake levels for pigs supplemented with an antibiotic.

In agreement with the studies of Hale & Newton (1979), Scheuermann (1993) and Kornegay & Risley (1996) the supplementation of a probiotic did not improve ($P<0.05$) the dry matter digestibility coefficient over the control treatment. However, the supplementation of EM at an inclusion level of 2% and the antibiotic did improve ($P<0.05$) dry matter digestibility over the inclusion level of 3% EM. The dry matter coefficient of this study agrees with the coefficients reported by Hale & Newton (1979).

Only small differences between the dry matter and apparent energy digestibility coefficients were recorded, and this is in agreement with the study of Kemm et al (1971). The significantly higher apparent energy digestion of the EM 2% treatment is in agreement with the significantly higher dry matter digestibility coefficient of this treatment over the EM 3%. The EM 2% treatment also had a higher ($P<0.05$) metabolizable energy value

than the EM 3% and control treatments. On the other hand, Scheuermann (1993) did not find a significant difference between different levels of a bacillus probiotic on the metabolizable energy values. According to Jonsson & Conway (1992) the digestibility of some nutrients may increase when the dosage levels are varied. In contrast with Scheuermann (1993), the supplementation of a mix culture probiotic at an inclusion level of 2% did improve the metabolizable energy digestion over the control treatments in this study. The EM 2% treatment, however, also had an improved ME/DE ratio over the control treatment. But, no significant differences were recorded between the EM 2% and control treatments for the fecal or the urine energy excretion.

In contrast with the reports by Hale & Newton (1979) and Kornegay & Risley (1996) the supplementation of a probiotic did influence the digestion coefficient of nitrogen in this study. Both the EM 1% and EM 2% treatments had a higher ($P<0.05$) digestion coefficient for N than the control treatment. These results agree with the results obtained by Maxwell et al. (1983) as cited by Kornegay & Risley (1996). The antibiotic treatment had a higher ($P<0.05$) N digestibility than both the control and EM 3% treatments. The slightly increased N intake of the antibiotic over the other treatments did not increased urinary N loss significantly. This is in accordance with the results of Scheuermann (1993).

In agreement with the results of this study, Scheuermann (1993) reported enhanced N retention as a percentage of N intake for growing pigs supplemented with a bacillus probiotic (46.1 vs. 48.7 and 48.2 % respectively for the control and different levels of bacillus probiotic). In this study the EM 2% treatment had a significantly higher retention than the control treatment. In contrast with these results Hale & Newton (1979) and

Kornegay & Risley (1996) did not find any significant improvement in N retention due to the supplementation of a probiotic in the diet of growing pigs. Mohan et al. (1996) recorded in broiler studies a significant difference (50.0 % vs. 48.4 %) in N retention with the addition of probiotics at 75 mg/kg feed in comparison with the control treatment. In spite the supplementation of a probiotic at 100mg/kg feed caused only a marginal increase (49.3 %) in N retention.

This improvement in ME MJ/kg and N balance as % of N intake by the EM 2% treatment over the control treatment is clearly reflected in the growth study (Chapter 2) where the EM 2% treatment had a lower, although not significant, mean feed intake and overall feed conversion than the control. In fact the EM 2% treatment had a lower feed intake and feed conversion than all the other treatments. This was however not statistically significant.

Although the antibiotic treatment had a significantly higher crude fibre intake than all the other treatments, the digestibility coefficient of this treatment was higher ($P<0.05$) than the coefficients for both the EM 2% and EM 3% treatments. These results are in contrast with the results of Hale & Newton (1979) who found that the addition of lactobacillus in the diets of the growing pig did improve crude fibre digestion (39.9 vs. 45.6). In this study the addition of a mix culture probiotic did not improve the crude fibre digestion. Kornegay & Risley (1996) could not found any effect of the addition of a bacillus probiotic to the diets of growing pigs on ADF and NDF digestion. According to Thacker (1988) the supplementation of probiotics may decrease carbohydrate utilization. The administration of a bacillus probiotic by Kornegay & Risley (1996) did not affect the pH

and the concentrations of lactic acid, ammonia or volatile fatty acids in cannulated pigs. It must be kept in mind that any digestive contribution by the micro flora to the nutrient requirements of the host must be balanced against losses resulting from dietary components becoming bound in microbial cells and voided in the feces (Sissons 1989).

In contrast with the results of Hale & Newton (1979) who reported no effect of probiotic supplementation on the ether extract (68.9 vs. 68.3), the results of this study show that the digestibility of ether extract was improved by inclusion of EM 1% and EM 2% over both the control and EM 3% treatments. However, the antibiotic treatment also enhanced ether extract digestibility over the control and EM 3% treatments. According to Sissons (1989) the administration of antibiotics improves fat digestibility in growing pigs. This effect is linked to an improve fat absorption in the hindgut, suggesting that fat metabolizing bacteria reduce the availability of dietary lipids to the host. It is proposed that microbial hydrogenation could increase the amounts of stearic acid, which is less well absorbed than unsaturated fatty acid or that the impaired lipid absorption could also be due to the deconjugation of bile acids in the gut (Visek 1979).

Hale & Newton (1979) and Kornegay & Risley (1996) could not found any improvement in the ash absorption. In contrast the results of this study shows a negative effect of the supplementation of a mix culture probiotic on the absorbability of the ash component. The EM 1% treatment had significant lower ash absorbability than the EM 2%, control and antibiotic treatments.

Conclusion

The hypothesis that neither EM nor a commercial antibiotic, did not improve nutrient digestibility and N retention could be rejected in accordance with the results obtained in this study. The addition of EM at an inclusion level of 2% improved both energy and nitrogen digestion. The fact that the EM 2% treatment had a significant higher metabolizable energy together with significant higher dry matter and ether extract digestibility than the EM 3% treatment show that the inclusion level of EM at 3% may be too high. This is supported by the fact that the antibiotic had significant higher dry matter and ether extract than the EM 3% treatment. However, except for ether extract the antibiotic and EM 1% treatments failed to improve nutrient digestibilities over the control treatment. It can therefore be concluded from the results of this study, that EM can be included in the diet of growing pigs at an inclusion level of two- percent Bokhasi. This may improve energy and nitrogen digestibility. However, more research needs to be done to determine the consistency of the results of this study.

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Chapter 4 Critical Evaluation

Study

Due to the complexity of probiotics in general, the mode of action of EM could not be determined. With future studies more emphasis could be placed on the mode of action of probiotics. In this study I could only be speculate about the mode of action where significant results were obtained. The approach was to give a multi culture probiotic from weaning until slaughter to get an overall interpretation of the effect of EM on the performance of the pig. The digestibility study was done to determine whether an increased performance could be directly related to an increase in nutrient digestibilities. It is evident that this was not the case in this study.

The approach in this study was to use a continuous dose system. However in future if the establishment of probiotic organisms could be determined single dose system could be followed. The storing method was standardized in accordance with the regulation of the suppliers of EM. With future studies more research can be done on the method of storing of probiotics. The method of storing will contribute to the viability of the microorganism within the probiotic. This however, will be determined by the type of probiotics used and the microbial composition of the probiotic. In future if maximum viability is the main objective, the specific probiotic may be stored under conditions similar to the optimum condition for survival of the specific microorganisms within the probiotic. These conditions may include; temperature, redoxspotensial, amount of light, pH and osmolarity.

In this study animals were never challenged with conditions similar to commercial conditions. In future studies the animals could be challenged with stress factors like temperature, crowding, disease and lower nutrient levels in diets to obtain more positive results regarding probiotics. Information on the influence of probiotics on carcass quality and the lactating sow could also be valuable.

General

When a MSc. study is undertaken the head of the department should give his written approval of the proposed protocol before any animals ect. are purchased.
As soon as a promoter accepts students he must be aware that he commits him self for the duration of this specific study. It is unacceptable that a promoter is allowed to leave the specific faculty when the specific study is not been completed yet.

This study was supervisor driven. The fact that the promoter (Dr.G.A.Smith) left university before the study was completed was detrimental to both the student and the study because the two supervisors did not share their enthusiasm for the product that was been tested. In addition it created a lot of communication problems between the student and the new promoter and this led to valuable time being lost.

Problems encountered during the study

- Funds were not sufficient for the proposed study. In the proposed protocol the pig would be bought from a producer and kept for the 150 day after which they would be slaughtered for carcass analysis. An additional phase whereby the performance of lactating sows was to be measured could not be implemented due to a lack of funds.
- During the whole of the experimental phase problems were encountered with labour. The student and a fellow student repaired all the required equipment i.e. digestibility crates and housing pens. The student and a fellow student recorded all parameters.
- Lack of facilities prevented the preparation of experimental diets on a daily basis to ensure the freshness of the enclosed product. No heating facility was available in the pig grower house.
- The laboratory and equipment were not always accessible for experimental analysis
- The statistical analysis were prolonged due to the fact that both statisticians had too many clients to satisfy

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In spite of all this I declare this study complete.

GERHARD PRETORIUS

DATE