A COMPARISON OF PHYSICAL CHARACTERISTICS OF PINE SHAVINGS, BIOSECURE PINE SHAVINGS AND SUNFLOWER HULLS AS LITTER MATERIAL AND ITS INFLUENCE ON BROILER PERFORMANCE

by

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DECLARATION

I, Mareli Smalberger, declare that this dissertation which I hereby submit for the degree of MSc(Agric): Animal Nutrition at the University of Pretoria is my own work and has not been previously submitted by me for a degree at this or any other tertiary institution.

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Date

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LIST OF ABBREVIATIONS

ADF	Acid detergent fibre
ADG	Average daily gain
AIAO	All-in, all-out
AOAC	Association of Official Analytical Chemists
BD	Bulk density
BS	Biosecure shavings
BW	Body weight
CP	Crude protein
DM	Dry matter
EE	Ether extract
FCR	Feed conversion ratio
FPD	Footpad dermatitis
GIT	Gastro-intestinal tract
NE	Necrotic enteritis
NSP	Non-starch polysaccharides
PEF	Production efficiency factor
PS	Pine shavings
SH	Sunflower hulls
WHC	Water-holding capacity
WRC	Water-releasing capacity

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ABSTRACT

This study was conducted to investigate three different litter materials, namely pine shavings (PS), bio-secure, fumigated pine shavings (BS) and sunflower hulls (SH) on their physical characteristics and how this influenced broiler performance parameters. The physical characteristics examined included bulk density (BD), pH, water-holding capacity (WHC), litter caking, proximate laboratory analysis of the litter materials and propensity to litter beetle infestation. Broiler performance was measured by production parameters: average daily gain (ADG), feed conversion ratio (FCR), production efficiency factor (PEF), slaughter weight, kilograms of broilers produced per m^2, mortalities and total feed consumed. Broiler gut development (weight and length) and footpad dermatitis scoring were also investigated. The results of this study revealed that litter converged toward similar physical characteristics ($P > 0.05$) at the end of a production cycle due to addition of feed, feathers and excreta. Broilers that had access to litter materials consumed their litter which was evident in the increased acid detergent fibre (ADF) levels found in gizzard contents versus feed. This led to improved gut development in the case of SH which translated to improved ($P < 0.05$) ADG, kg/m^2 and slaughter weight. The SH contained more nutrients ($P < 0.05$) based on proximate analysis as compared to the other litter types. However, improvements seen with SH did not alter ($P > 0.05$) the commercially measured figures of PEF and FCR. The SH was associated with an insect (*Tribolium castaneum*) not often associated with poultry houses which could hold as yet unidentified disadvantages to producers. The SH had the lowest overall *Alphitobius diaperinus* activity ($P < 0.0001$) which may offset disadvantages. Biosecure pine shavings had no superior effect when compared to PS ($P > 0.05$). Management of litter remains an important part of achieving production targets, irrespective of the litter type used.

Key words: Gut development, litter, litter physical characteristics, pine shavings, sunflower hulls

CHAPTER 1

1.1 Introduction

There are three major factors driving financial return in poultry production, namely: body weight gain, feed conversion, and mortality rate. To improve broiler performance, production aspects such as chick and feed quality commonly receive focus, while litter quality and bedding material are often given less attention. However, previous research has indicated that bedding source and litter quality have a significant impact on the attainment of live performance targets, which ultimately impacts the profitability of production.

Litter is defined as the bedding material together with the excreta, feathers and wasted feed and water (Ritz *et al.*, 2009). The choice of bedding by producers is frequently driven by what is readily available in the area. In South Africa, the types of bedding used for poultry production include wood shavings, sawdust, wheat straw, sunflower and peanut hulls (Jordaan, 2004). Preferred bedding sources include pine shavings and pine sawdust, but these are becoming scarce and increasingly expensive (Ross Broiler Management Guide, 2014). Sunflower hulls are a cheap source of litter, but are only seasonally available, have a lower absorptive capacity, and may be prone to litter beetle infestation. There is also little scientific research that has evaluated sunflower hulls as a bedding source, when compared to wood shavings. In spite of this, sunflower hulls are widely used as a bedding source in South Africa (Jordaan, 2004).

The characteristics of good poultry bedding include constant availability, low cost, absence of toxic substances, the ability to absorb and release moisture rapidly, and low initial moisture content (Bilgili *et al.*, 2009). Litter serves several purposes for the welfare of the birds, such as insulating chicks from the floor of the house, diluting faecal material, and providing the birds with a surface to scratch, peck and perform dust baths (Ritz *et al.*, 2009). The relationship between broilers and litter is an inter-related web, with various aspects of the litter impacting all spheres of the broilers' lives.

The type of bedding and resultant litter conditions are also important for broiler welfare, due to its influence on the incidence of footpad dermatitis. Footpad dermatitis occur as burns on the feet of broilers, since continuous contact exists between the footpads of the birds and the litter. Footpad dermatitis can develop in less than a week under suboptimal litter conditions. Litter that contains more than 65% moisture promotes the growth of bacteria and moulds (Ritz *et al.*, 2009). Litter moisture levels should be maintained between 20 and 30 percent. Caked litter reduces the moisture-absorbing capacity, and prevents moisture from evaporating (Collett, 2012).

Consistently available, good quality bedding from a reliable bedding supplier is imperative for optimal broiler welfare, nutrient digestion, and performance (Ross Broiler Management Guide, 2012). Litter from unverified sources may contain parasites, such as the litter beetle (*Alphitobius diaperinus*), a common South African parasite. Litter beetles can cause large-scale destruction and result in huge maintenance costs, because the beetles tunnel through the insulation panels of environmentally controlled housing, destroying insulation capacity.

The type of bedding used has also been shown to influence gizzard development and digestive function (González-Alvarado *et al.*, 2008; Amerah *et al.*, 2009). Birds consuming bedding with a coarse structure like pine shavings had improved gizzard development, which could aid in better digestion, and thus, better feed conversion (Amerah *et al.*, 2009). Subsequently, increased reverse peristalsis can also alter intestinal microbiota composition, potentially increasing the amount of nutrients available to the chicken, suppressing pathogenic bacteria by means of competition and altering gut morphology (Torok *et al.*, 2009a; Torok *et al.*, 2011). Due to consumption of the litter, up to 4% of a broiler's diet can consist of their litter (Malone *et al.*, 1983).

Several studies have found no statistically significant differences in broiler feed conversion and performance among different bedding sources in deep litter conditions (Bilgili *et al.*, 2009, Torok *et al.*, 2009a; Toghyani *et al.*, 2010, Garcia *et al.*, 2012a). However, there is also comparatively little research in this area under more commercial conditions, using complete cleanout of litter at the end of each cycle, as practiced in South Africa. In the competitive commercial industry, even small differences could lead to substantial cost savings (Swain & Sundaram, 2000).

1.2 Aim

The aim of the project was to evaluate the effect of bedding source on performance, mortality, microbial load and welfare of broilers under commercial conditions. Three types of commonly used bedding materials in South Africa were investigated in this project, namely bio-secure, fumigated virgin pine shavings, non-chemically treated pine shavings, and sunflower hulls.

1.3 Objectives

The three abovementioned litter sources were compared in terms of:

- physical characteristics of litter (bulk density, pH, litter caking, litter microbes, water-holding capacity, water-releasing capacity, litter beetle counts);
- laboratory proximate analysis of litter (dry matter, ash, ether extract, acid-detergent fibre, crude protein)
- footpad dermatitis occurrence in broilers;
- production parameters (total feed consumed, average daily gain (ADG), feed conversion ratio (FCR), production efficiency factor (PEF), mortality, broiler meat yield/m^2, slaughter weight); and
- broiler gut development (proventriculus and gizzard weight, dimensions and acid-detergent fibre content; intestinal weight and length).

CHAPTER 2

Literature Review

2.1 Introduction

Poultry litter is defined as the bedding material together with the excreta, feathers and wasted feed and water (Ritz *et al.*, 2009). Optimal litter conditions can have a significant impact on the attainment of live performance targets, such as body weight gain, feed conversion ratio, mortality percentage, and production efficiency factor, ultimately impacting the profitability of production. Despite this, litter quality and bedding material are often neglected by producers in favour of production aspects such as chick and feed quality.

Producers often utilise bedding materials based solely on constancy of availability in the region. Types of bedding used for poultry production in South Africa include wood shavings, sawdust, wheat straw, sunflower and peanut hulls (Jordaan, 2004). Pine shavings and sawdust are seen as ideal bedding sources to use, but are becoming scarce and increasingly expensive (Ritz *et al.*, 2009; Ross Broiler Management Guide, 2014). Sunflower hulls are a cheap source of bedding, but are only seasonally available, and research is lacking regarding its use in comparison to other bedding sources, although it is used widely (Jordaan, 2004).

There are several characteristics that constitute a high quality poultry bedding source. Some characteristics include low cost, constant availability, low initial moisture content, and the ability to absorb and release moisture rapidly. It must also be lightweight, non-toxic and should not taint the meat (Bilgili *et al.*, 2009). Litter serves several purposes for the welfare of the birds, such as providing the birds with a surface to scratch, peck and perform dust baths, insulating chicks from the floor of the house and diluting faecal material (Ritz *et al.*, 2009). It is imperative when evaluating different litter types that not only the material itself be evaluated, but also the management of the litter, especially when controlling litter moisture (Angelo *et al.*, 1997; Toghyani *et al.*, 2010).

Poultry litter legislature recommends friable litter, that does not become caked and hard. Wet litter should be removed immediately, with an investigation into the cause thereof (Lister, 2009; SAPA code of practice, 2012). Excessively dry litter becomes dusty and may lead to respiratory diseases (Torok *et al.*, 2009a). Litter pathogens may enter the gastro-intestinal tract (GIT) of birds and cause enteric disease. Several physical characteristics of litter adversely affect broiler performance in a number of ways, which will be reviewed in detail. Feed conversion, broiler performance, gizzard development and the gut microbial composition of young broilers may be influenced by litter type (Lu *et al.*, 2003b; Ali et al., 2009; Bilgili *et al.*, 2009; Torok *et al.*, 2009a; Toghyani *et al.*, 2010; Garcia *et al.*, 2012a). The role of ammonia regarding litter type and broiler performance is also investigated, since it is the most pronounced and damaging gas emitted by litter. Other topics touched on in this literature review include other uses of poultry litter, such as recycling, use as cattle feed and fertiliser.

2.2 Effects of litter on broiler welfare

General welfare

In commercial broiler production, broiler welfare may be affected by bird growth rate, light intensity, nutrition, feeding systems, environmental control, stocking density and litter depth (Shao *et al.*, 2015). Litter serves several purposes for the birds, such as insulating chicks from the floor of the house, diluting faecal material, and providing the birds with a surface to peck, scratch and perform dust baths (Ritz *et al.*, 2009). These are an integral part of the bird welfare, and form part of the five freedoms of production animals, according to the Brambell Report (1965), namely: freedom from hunger and thirst; freedom from pain and disease; freedom from discomfort; freedom to express natural behaviour; and freedom from fear.

The degree of feathering present on the birds is a subject sparking significant welfare concerns. Garcia *et al.* (2012b) found no significant differences in the rate of feathering of birds reared on different litter types, but found that females start feathering earlier than do males. It is important in warm climates that feathering is less extensive, which improves heat dissipation, but poor feathering also exposes the birds to lesions. In a study (Edens *et al.*, 2000) comparing selenium sources on different litter types, feathering was affected by litter type. Recycled paper litter treated with 4.5% boric acid caused reduced feathering in both spring and summer rearing for unknown reasons, but when the effect of boric acid was diluted with the addition of 50% wood shavings, the depression in feathering was reduced. Feathering scores were also improved ($P < 0.05$) when wood shavings and untreated newspaper were used, as compared to recycled paper litter. Boric acid is commonly used to control litter beetles (*Alphitobius diaperinus*) on recycled litter material (Dufour *et al.*, 1992). In a study related to boric acid toxicosis (Dufour *et al.*, 1992), day-old broilers were fed 0, 2500ppm and 5000ppm boric acid for two weeks and a dose-related reduction in feathering was observed.

In terms of bird behaviour, it was found that sand is the preferred litter material for birds to exhibit normal behaviour, such as pecking and performing dust baths. Wood shavings also allowed broilers to continue this behaviour (Bilgili *et al.*, 2009; Toghyani *et al.*, 2010). Stocking density has a large effect on the broiler welfare, as an increase in the number of broilers puts more pressure on the litter, with more excrement leading to higher litter moisture content. When stocking density exceeds 40-45 kg/m^2 litter management becomes critical, and the incidences of footpad dermatitis and breast blisters are likely to increase (Dozier *et al.*, 2006). Stocking densities that provide less than 0.05m^2 per bird, may have a negative effect on performance from week four onwards (Zhang *et al.*, 2011).

Footpad dermatitis & breast blisters

Plantar pododermatitis, also known as footpad dermatitis (FPD), is a common management-related condition, which occurs globally in poultry houses. It normally presents as superficial lesions on broilers' footpads, but may cause pain and discomfort to the birds when deep ulcers form (Martland, 1985; Bilgili *et al.*, 2009). Footpad dermatitis can develop in less than a week under suboptimal litter conditions. The early stages of FPD present as lesions on the skin, surrounded by reddening of the skin, followed by discolouration of the skin on the footpads. Histologically, hyperkeratosis and necrosis of the epidermis can be seen. As the infection progresses, inflammation and ulceration of the subcutaneous tissue occur (Greene *et al.*, 1985). Pus forms on the plantar surface, which cause excreta and litter material to adhere to the footpads and form crusts, which lead to secondary bacterial infections. The infection may clear up if the litter is subject to major improvements, however, broilers are often sent to slaughter before the footpads are fully healed (Martland, 1985). Other manifestations of the disease occur as hock burn and breast blisters, where similar symptoms may be observed (Greene *et al.*, 1985). Affected birds have a challenged immune system that causes their health to deteriorate, and they find it painful to walk. These birds will become more lethargic and consume less feed and water, leading to reduced performance and dehydration.

The most critical factor for the incidence of FPD is the type, quantity and quality of litter material since continuous contact exists between the footpads of the birds and the litter. Litter deterioration inevitably leads to increased incidence of FPD (Cengiz *et al.*, 2011). All factors relating to wet litter will also have an effect on FPD, since litter with a low water absorption capacity, which is prone to caking, will increase FPD occurrence in a flock. Litter with abrasive edges may nick the epidermis and once the skin is broken, the footpads are exposed to litter bacteria, which may cause FPD, with the result that large litter particles are not desired (Cengiz *et al.*, 2011). The litter should be deep enough to reduce friction, absorb impact and insulate the broilers from the house floor (Garcia *et al.*, 2012b). If these requirements are not met, the incidence of FPD in the house will rise.

There are also other factors that will influence the incidence of FPD. If birds get diarrhoea from either infectious or non-infectious causes, litter will be wet; leading to higher microbial loads in the litter, and the prevalence of FPD might increase. The risk of FPD will be heightened when birds lie down for long periods, for instance, when a lighting programme with long dark periods is followed, or if there are leg problems within the flock (Berg, 2004). High stocking density will also increase the incidence of FPD. When gender is considered, males tend to have a higher incidence of FPD and breast blisters than females do, because males are heavier and become feathered later (Toghyani *et al.*, 2010; Garcia *et al.*, 2012b). Holistic management of wet litter is the best preventative measure for FPD. For the evaluation of FPD in a broiler house, scoring of the footpads is the most sensitive indicator of FPD, and is preferred above examining hock or breast blisters. Several scoring systems have been utilised for evaluating the severity of FPD lesions (Ekstrand *et al.*, 1998; Nagaraj *et al.*, 2007; Hocking *et al.*, 2008; El-Wahab *et al.*, 2010). Scoring ranges from a three-point score to a seven-point score, which visually classifies the severity of lesions. In the example below, footpads were scored in three classes: class 0 = no signs of FPD; class 1 = mild lesions - discolouration and hyperkeratosis may be seen; class 2 = severe lesions with ulcers and scabs (Ekstrand *et al.*, 1998; El-Wahab *et al.*, 2010). From this basis, scores can also be modified to take the depth of the lesion or presence of additional lesions into account.

Figure 2.1. Footpad dermatitis scoring system (El-Wahab et al., 2010)

2.3 Effects of litter on broiler performance

Effect of litter on gizzard development

The gizzard is a muscular organ with the ability to grind digesta particles to a fine size. In the anterior organ, the proventriculus, hydrochloric acid and pepsinogen are secreted, but due to its small size, the retention time in the proventriculus is very short, and most of these secretions are utilised in the gizzard. The low pH in the gizzard originates from the proventriculus secretions, which aid in the digestion of particles. Its muscular structure and different contractions allow it to grind and crush digesta (Svihus, 2011).

Only particles below the threshold size of 0.5-1.5 mm are permitted to enter the duodenum (Ferrando *et al.*, 1987), however, most particles are no larger than 0.1 mm (Amerah & Ravindran, 2008). In birds consuming diets with more structural fibre, digesta particle size in the duodenum is smaller than in birds with a lower dietary fibre content, which may be due to increased grinding in the gizzard. Larger duodenal particles have a smaller surface area available for absorption, and could contain starch granules trapped within, which could lead to reduced absorption (Hetland *et al.*, 2003; Amerah *et al.*, 2009). When larger particles are consumed, grinding action of gizzard contents is increased in order for particles to reach threshold size. The larger particle size is correlated to gizzard development, with particles larger than 1 mm exerting a positive effect (Nir *et al.*, 1997), where the highest correlation was found when particles were larger than 2.8 mm (Svihus, 2004).

Broiler diets contain very little fibre, due to their high energy density. It was believed that dietary fibre is a diluent of the diet but later found that increased amounts of fibre in the diet lead to improved gut development (Mateos *et al.*, 2012). Bedding materials including sawdust, wood chips, straw and wood shavings, are mostly composed of insoluble NSP and lignocellulose (Choct, 2009). Different litter types and different diets have been found to alter broilers' gizzard development (Amerah *et al.*, 2008; González-Alvarado *et al.*, 2008). Broilers reared on wire floors responded well to fibre inclusions in their diets. When 3% oat hulls were included, gizzard size increased by 35% (Hetland & Svihus, 2001), but 3% soy hulls did not stimulate gizzard development. The inclusion of 6% wood shavings of 1-2 mm in size increased gizzard size by 39% (Amerah *et al.*, 2009). Bilgili *et al.* (1999) found that birds reared on sand had significantly ($P < 0.05$) smaller gizzard sizes, lower gizzard weight, and contained more digesta than birds reared on pine shavings. Some studies disagree on whether litter type has an effect on gut development. No differences were observed in 42-day performance ($P > 0.05$) or gut microflora ($P > 0.05$) of broilers reared on rice hulls, softwood sawdust, pine shavings, reused single batch litter, hardwood sawdust, shredded paper or chopped straw, however, differences did occur at 14 days of age (Ali *et al.*, 2009). Broilers reared on wood shavings had larger gizzards than birds reared on rice hulls or paper roll, although the findings in this regard were not significant ($P > 0.05$), according to Toghyani *et al.* (2010).

Broilers respond rapidly to inclusion levels of structural fibre in the diet with reports of gizzard size increase of up to 100% of its original size (Gabriel *et al.*, 2003; Sacranie, 2010). Gizzard volume often increased by a larger magnitude than gizzard size, which leads to a larger gizzard holding capacity being established (Amerah *et al.*, 2008; Amerah *et al.*, 2009). Up to 4% of a broiler's total consumption can consist of the litter (Malone *et al.*, 1983) without having an effect on feed conversion efficiency (Hetland *et al.*, 2003). High consumption levels (more than 10%) of structural fibre such as litter may slow down digesta passage rate, which in turn lowers the level of intake, leading to a reduction of feed efficiency and performance (Hetland *et al.*, 2003).

In battery rearing conditions, or on floors without litter, chicks have been found with distended proventriculi and proportionately under-developed gizzards, due to a lack of fibre in the diet. Birds reared on litter displayed larger gizzards and no proventriculus distention. When small amounts of shavings were added to the enclosures of birds in the former situation, the problem was corrected (Riddell, 1976). Laying hens on a mashed diet have been observed to consume feathers when no other fibre source was available (Hetland & Svihus, 2007).

Effects of litter on gut microbiota

The gut microbiome is extremely complex, harbouring around 700 species of bacteria and 20 hormones. In addition, 20% of the body's energy is utilised by the gut (Choct, 2009).

The intestinal microbiota is crucial for the well-being of broiler chickens, because it is the body's largest immune organ (Kraehenbuhl & Neutra, 1992). It performs various roles such as increasing the number of nutrients available to the chicken, suppressing pathogenic bacteria by means of competition and altering gut morphology (Mead, 2000). Gastrointestinal microbiota contain a mix of protozoa, fungi and bacteria, with bacteria dominating the gut (Gabriel *et al.*, 2006). The microbial composition is determined, to a large extent, by the diet of the broiler, but also by its environment including litter type, litter conditions, and climate as well as hatching conditions.

The intestinal tract is sterile at hatch, but shortly after hatch, microbes start colonising the intestinal tract and a large diversity of microbiota proliferate. Microbial density in the intestine increases from the proximal end to the distal end. Each section of the intestine houses a sub-population of microbes which differs between sections, while still maintaining functional continuity in order to perform functions such as carbohydrate breakdown, fermentation, and vitamin synthesis (Hooper *et al.*, 2002). The host and microbes have a symbiotic relationship, since the microbes provide the host with nutrients that would otherwise be unavailable to the host (Savage, 1986) while the host provides an ideal habitat to the microbes. Host and microbe also compete for certain nutrients, thus it is important for microbial populations to be controlled to prevent the proliferation of potentially pathogenic microbes. Obviously, gut health is paramount to ensuring animal health (Choct, 2009).

Younger birds have a larger variety in gut microbiota, since the microbial composition is not yet matured, and is thus unstable (Torok *et al.*, 2009a). The ileal composition undergoes three major shifts between day 3-17, regardless of diet, with these shifts observed around day 3-5, day 5-12 and day 12-17 involving mostly *Lactobacillus* species (Torok *et al.*, 2009b). Most bacteria of the chicken caecum that can be cultured are obligate anaerobes, and originate from a large number of bacterial strains. Many bacteria cannot be cultured, and estimates are that only 10 to 60 % of bacteria can be recovered in culture (Barnes *et al.*, 1972a; Barnes & Impey, 1972). 16S-rRNA clone libraries were developed and compared to existing GenBank sequences to perform more balanced views of the ileal and caecal microbial composition of broilers, but the amplification methods may be biased towards particular strains (Zhu *et al.*, 2002). Families of similar gut microbial strains have been found across several species, including ruminants, hindgut fermenters and humans, but specific strains are adapted to survive solely in the chicken intestinal tract (Edwards *et al.*, 2008). Thirteen strains of predominating bacteria found in the ileum and caecum overlapped, but their abundance differed largely. Figure 2.2 depicts a representation of the bacterial composition of the ileum and caecum (Adapted from Lu *et al.*, 2003b).

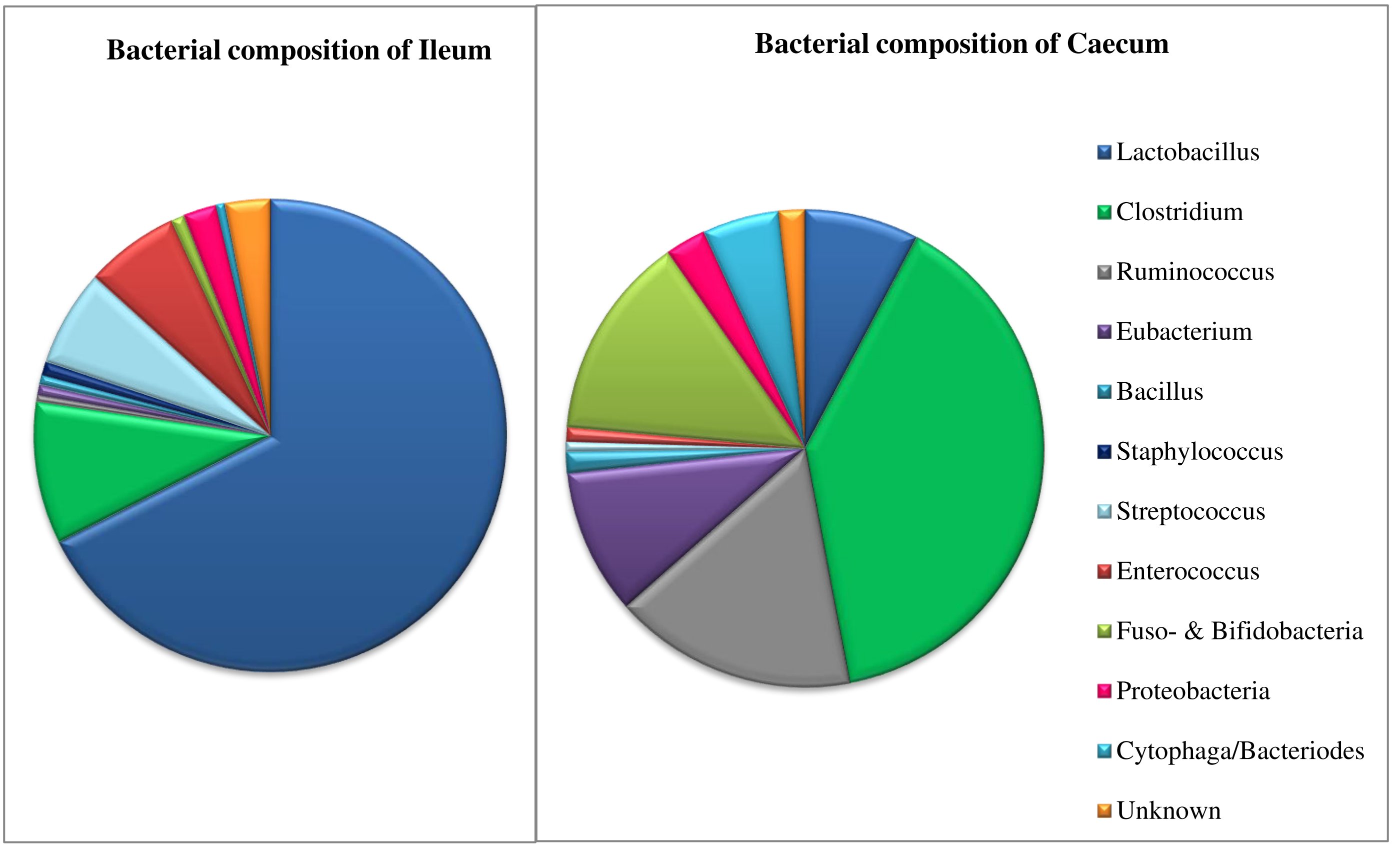

Figure 2.2. Bacterial composition of the chicken intestinal tract (Adapted from Lu *et al.,* 2003b)

Up to 4% of a broiler's total intake can consist of the litter (Malone *et al.*, 1983). Different litter types and different diets have been found to alter broilers' gut microbial composition (Torok *et al.*, 2009a; Torok *et al.*, 2011). The effect of different litter types on chick caecal microbes was evident with chicks reared on reused litter exhibiting the lowest caecal microbial counts. Both the Ross and Cobb management guides do not recommend re-using litter, since it increases the amount of potentially pathogenic microbes to which the birds are exposed. Re-used litter could also be linked to lower live weight, increased morbidity, and poorer feed conversion of broilers at 14 days of age, but these differences diminish at 28 days of age (Torok *et al.*, 2009a). When various new litter types were compared, caecal microbial composition differed ($P < 0.05$) at 14 days of age between chickens raised on rice hulls, as compared with softwood sawdust, hardwood sawdust, or shredded paper; and chickens raised on hardwood sawdust, as compared with shredded paper, or softwood sawdust. At 28 days of age, differences ($P < 0.05$) in caecal microbial composition were found with rice hulls, as compared with shredded paper, or chopped straw; and birds raised on softwood sawdust, as compared with chopped straw (Torok *et al.*, 2009a). These differences may become important in managing gut health without in-feed antibiotics to reduce disease and increase feed efficiency (Torok *et al.*, 2009a).

Litter effects on feed conversion and performance

Several studies have found no significant effect of litter type on broiler feed conversion and performance (Bilgili *et al.*, 2009; Torok *et al.*, 2009a; Toghyani *et al.*, 2010; Garcia *et al.*, 2012a). The feed conversion of birds reared on sand, wood shavings, rice hulls, sawdust, shredded paper, chopped straw, paper roll or no litter was not affected by litter type (Torok *et al.*, 2009a; Toghyani *et al.*, 2010). According to the litter material selection criteria drawn up for producers in Brazil, broiler performance has a relatively small effect on the ultimate selection of litter material. Other factors, such as the possibility of reuse, ease of handling, and availability played a proportionately larger role (Garcia *et al.*, 2012a). In the competitive commercial industry, even small differences could lead to substantial cost savings.

When different diets are fed, performance differences do exist, but this is probably a function of the different feeds rather than the litter (Swick *et al.,* 2012). In a comparison between several litter types, the 14 day live weight of broilers was lower ($P < 0.05$) with broilers reared on reused litter, compared to the weight of those reared on hardwood sawdust and chopped straw. Feed intake at 14 days also differed ($P < 0.05$) with broilers reared on hardwood sawdust and chopped straw consuming more feed than those reared on shredded paper or reused litter. When comparing live weight and feed consumption, the performance on pine shavings was in between these two extremes. No differences were found at 28 days of age (Torok *et al.*, 2009a). Broiler performance values did not differ ($P > 0.05$) when reared on rice husks, sawdust or coir dust, but feed conversion ratio (g feed/g gain) differed between 2.50 and 2.58 on rice husks and coir dust respectively, a difference that may hold a financial implication (Swain & Sundaram, 2000).

2.4 Physical characteristics of litter

Bedding source

A reliable bedding supplier which can supply consistently available, good quality bedding is imperative for optimal performance (Ross Broiler Management Guide, 2012). The characteristics of good poultry bedding include constant availability, low cost, the ability to absorb and release moisture rapidly and low initial moisture content. It must also be lightweight, non-toxic and should not taint the meat (Bilgili *et al.*, 2009). If a chemical is present in the litter, it has a high probability of ending up in the broiler meat. This is necessarily an important consideration, especially when using by-products from other industries (Ritz *et al.*, 2009).

Litter from unverified sources may contain parasites. A common litter parasite in South Africa is the litter beetle, also known as the lesser mealworm, or darkling beetle (*Alphitobius diaperinus*). This beetle is present in litter from unverified sources, where re-used litter also creates a favourable environment for beetle development. Litter beetles can cause large-scale destruction by tunnelling through the insulation panels of environmentally controlled housing and destroying the insulation capacity. The cost involved in replacing the panels every few years amounts to millions of rands for poultry producers. In order to implement a pest control management programme, it is necessary to understand the biology of the beetles and the conditions they require to proliferate. They are omnivorous and may feed on litter, grain, as well as weak and dead chicks (Harris, 1966). The life cycle of the beetle takes 89 days at 22 °C and only 26 days at 31 °C, and adults may live approximately one year.

Various studies have been conducted on the environmental factors that aid the proliferation of litter beetles, the most pronounced of which are temperature and humidity (Wilson & Miner, 1969; Strother & Steelman, 2001; Chernaki-Leffer *et al.*, 2007). Beetle populations aggregate in damp areas, such as litter under leaking drinker nipples and form dense, localised populations. Almost no larvae developed at 9% moisture, but moisture levels of 15% seemed to coincide with the highest beetle production and development, according to Wilson & Miner (1969). Their experiment did not test beetle development under moisture levels of more than 15%, but indications are that higher moisture levels favour beetle development. Despins *et al.* (1989) agreed with this finding, stating that all life stages of the beetle preferred litter moisture levels of 30-40%, and started to migrate away from the litter when it reached 50-60 percent.

The optimal temperature for beetle eggs to develop into larvae and adult stage is around 32°C. At this temperature, development occurred at the fastest rate, and the largest proportion of eggs hatched. At 37°C mortalities occurred, but litter beetles thrive at lower temperatures than that. The lower temperature threshold for beetle development is 10°C (Wilson & Miner, 1969). These temperature and moisture thresholds indicate that poultry houses provide the optimal environment for beetles, and that their life cycle may be completed by the time a broiler production cycle is done. Since they migrate into the insulation panels, getting rid of them becomes a massive task. Studies about the spatial abundance of the beetles agreed that beetle levels increased throughout the sampling period (Strother & Steelman, 2001; Chernaki-Leffer *et al.*, 2007). Beetles seem to favour the middle, walls and back of the houses (Strother & Steelman, 2001). To monitor beetle numbers in the house, the use of Arends trap tubes are recommended, although it is time-consuming to count the larvae and adults (Safrit & Axtell, 1984; Arends, 1987; Camargo Neto *et al.*, 2006).

Litter beetles also act as reservoirs for other pathogens, such as Salmonella (Skov *et al.*, 2004). Bedding that has been kept in a bio-secure chain prior to placement in the house contains a smaller risk of being contaminated with litter beetles. After any disease challenge, proper fumigation and sterilisation of poultry houses is recommended, as well as ensuring more days open between production cycles. If fresh litter is placed in a poorly disinfected house, the pathogens will utilise the litter as a medium in which to proliferate (Skov *et al.*, 2004).

Particle size

Large litter particles may cause impaired absorption and increased litter compaction. Studies dating back to the 1950s (Aho *et al.*, 1955) indicate that particle sizes of 2–5 cm possess improved absorptive capacity over particle sizes of 7–10 cm when wood chips are used. Litter particle size needs to be taken into account when assessing incidence of FPD. Cengiz *et al.* (2011) suggested that 0.5 cm was an appropriate screening size for pine shavings. This study also emphasised that larger particle size led to an increase in FPD incidence as a result of the abrasive action of large particles with rough edges on the footpads. Lesions on the carcass could also be caused by abrasive edges on litter (Garcia *et al.*, 2012b).

Bedding with a small particle size is prone to dustiness and may cause irritation of the airways of the birds. Litter particle size tends to reduce as the cycle progresses since the constant movement of broilers can break up particles in non-caked areas (Garcês *et al.*, 2013). Chickens tend to find the smaller particle size preferable to large litter particles as demonstrated by a trial investigating the dust-bathing behaviour of layer hens. The hens' dustbathing behaviour increased as the trial progressed and the litter particle size decreased. The most pronounced effect on dustbathing was the size of the litter particles and not the depth of litter (Moesta *et al.*, 2008).

Litter depth

Litter should be deep enough to reduce friction, absorb impact and insulate the broilers from the house floor (Garcia *et al.*, 2012b). Litter should also be evenly spread out, since uneven spreading may lead to the restricted access of feed and water for young chicks, which consequently reduces flock uniformity (Ross Broiler Management Guide, 2014). Mizu *et al.* (1998) found no significant differences ($P > 0.05$) in Bangladeshi broiler performance at depths of 2, 3, 4 and 5 cm, respectively. However, Chinese research found significant differences ($P < 0.05$) among several parameters at litter depths of 4, 8, 12 and 16 cm. Body weight gain, daily weight gain and feed intake increased with increasing litter depth. In terms of immune organs, absolute and relative liver weight decreased with increasing body weight. Other immune organs (thymus and bursa of Fabricius) and welfare indicators, such as FPD and hock burn were not affected by litter depth (Shao *et al.*, 2015).

Shallow litter contains a higher level of moisture, which leads to a relatively larger bacterial community (Shao *et al.*, 2015). On the other hand, very thick litter (> 16 cm) may be unfavourable to performance, health and welfare since it has a higher dust content (Al-Homidan *et al.*, 2003). Table 1 gives general guidelines for optimal litter depth.

Table 2.1. Guidelines for optimal litter depths

	Type of litter	**Recommended depth**	**Source**
Sweden	General	0.5- 1 kg/m^2	Elwinger & Svensson (1996)
Brazil	General	0.1 m	Garcia *et al.*(2012b)
Ross guidelines	General	0.05 -0.1 m	Ross Broiler Management Guide (2014)
Cobb guidelines	Wood shavings & dry sawdust	0.025 m	Cobb broiler management guide (2012)
Cobb guidelines	Sunflower hulls	0.05 m	Cobb broiler management guide (2012)
-	General	0.05 m	Kocak (1978)

Litter caking

With the help of the codes of recommendations for the welfare of livestock, Lister (2009) established that poultry litter legislature approved of friable litter – litter that is not too sticky and caked, nor too dry and dusty. In South Africa, the SAPA code of practice (2012) recommends that litter should not become too caked and hard and that wet litter should be removed immediately with an investigation into the cause thereof.

Once litter becomes caked, the moisture absorbing capacity drops markedly. Caked, compacted litter prevents moisture from evaporating, thus litter turning is recommended in these instances to reduce the impact of compaction (Collett, 2012). Caked litter contains 1.5-2 times the amount of moisture than loose litter and moisture content varies more between cakes than different loose litter samples (Malone *et al.*, 1992; Coufal *et al.*, 2006). Litter caking is influenced by several factors, including ambient temperature, litter type, ventilation rate and drinker management (Coufal *et al.*, 2006).

Litter moisture

Wet litter promotes the growth of bacteria and moulds (Ritz *et al.*, 2009). Litter moisture levels should be maintained between 20 and 30 percent. Litter is seen as wet when litter moisture reaches 65 percent. Litter with more than 250 g/kg moisture has reduced insulating and water-holding capacity (Collett, 2012). Low initial moisture content is essential for maintaining better litter quality for longer, and ensuring that early growth retardation does not occur due to high litter moisture content (Cengiz *et al.*, 2011). Litter is aerated when broilers scratch and take dust baths, which creates an opportunity for moisture to be released. Aerobic bacterial activity also occurs and generates heat that accelerates moisture evaporation (Lister, 2009).

Wet litter can occur as a result of both non-infectious and infectious factors. Non-infectious factors span over a range of nutritional and management issues. Biosecurity needs to be up to standard to lower the pathogen risk for the birds. Drinker- and ventilation systems should also be inspected to ensure proper functioning (Lister, 2009). Litter moisture must be carefully monitored in winter when ventilation rates are at a minimum. Under these conditions, the primary objective of an environmentally controlled house is to remove excess moisture (Collett, 2012). A high mineral content, incorrect pH and total bacterial count in the drinking water will also lead to watery excretions (Lister, 2009). The stocking density must be appropriate for the type of housing in which the birds are housed. High stocking density will inevitably lead to excessive fouling of litter, which will increase moisture content. At these stocking rates, even small changes in moisture output of the chickens can lead to problems with wet litter.

Various dietary aspects can increase litter moisture, such as a high nitrogen content, which evidences a high or imbalanced protein diet. Water insoluble non-starch polysaccharides (NSPs), oxidised fats, over- or undercooked soya, and an increase in dietary cations (sodium, potassium, calcium and magnesium) are all potential culprits of poor quality litter (Kleyn, 2013). The rapid genetic gain in past decades has led to birds consuming more feed and water, and consequently excreting more faeces (Collett, 2012). Water quality can also alter the litter moisture and it is imperative to check the pH, minerals, and bacterial count (Lister, 2009).

Infectious factors include pathological conditions involving viruses, bacteria or other parasites, which can cause a variety of enteric diseases and intestinal disturbances. The most pronounced pathogens are coccidia of the type *Eimeria, Escherichia coli, Clostridium perfringens* and numerous *Salmonella* and *Campylobacter* species. When the intestinal lining becomes inflamed, it has a reduced capability to absorb nutrients. The balance of the gut microflora must also be maintained to ensure proper functioning of the gastro-intestinal tract (GIT; Kleyn, 2013).

Bulk density

Different bedding sources have differing weights, which may pose problems for transportation, as similar volumes of these sources are needed to cover the poultry house floors. For instance, sand is four times heavier than wood shavings (Garcês *et al.*, 2013). The amount of excreta, feed, feathers and water added to the bedding is independent of the source used, and according to Garcês *et al.* (2013) the average amount produced per bird is 896g. This is important to consider if the litter is sold as fertilizer or supplementary feed for ruminants (Mavimbela *et al.*, 1997; Van Ryssen, 2001; Garcês *et al.*, 2013).

Bulk density is the weight of a unit volume of a loose material (such as poultry litter) to the same volume of water. Bulk density generally increases as the cycle progresses due to added excreta, feed, feathers and water, and a reduction in particle size, which can increase bulk density by 2.4 times over the course of a production cycle (Garcês *et al.*, 2013). Bulk density may decrease if added debris has a lower density than the particles of the bedding source (Garcês *et al.*, 2013). Since the contribution of debris is independent of the litter source, the relative bulk density between sources may serve as an indication of the amount of compaction in the litter. However, this method is less conventional than litter caking scores and lacks research (Garcês *et al.*, 2013).

2.5 Effects of litter on disease incidence

Respiratory disease

Ascites is a metabolic disease caused by pulmonary arterial hypertension that is associated with various factors relating to the supply of oxygen to the tissues of the broilers (Sillau & Montalvo, 1982). Ascites normally occurs during the latter half of broiler rearing, when supply organs come under huge strain to support the heavily muscled broiler. Ascites is a result of the exceptional efficiency of modern genotypes, and causes visceral oedema. Contributing factors include high altitudes and environmental conditions. Hypoxia occurs more readily in areas high above sea level, and in winter where the temperature drops significantly (hypobaric hypoxia), where the atmospheric partial pressure of oxygen is lowered under these circumstances (Ruiz-Feria & Wideman, 2001).

Litter material that tends to become dusty, such as sawdust, are correlated with an increase in respiratory disease (Garcês *et al.*, 2013). Poor air quality with increased ammonia emissions and dustiness will irritate the airways and contribute to ascites. Good litter and drinker management are essential for managing ammonia levels (Kleyn, 2013), as discussed below (See the section on effects of litter on gas emissions, p. 18).

Potential gut pathogens

There is increased pressure from governmental organisations to ban the use of antibiotic growth promoters, due to a fear of extensive antibiotic resistance. It is uncertain as to how this will influence the gut microbiological population in modern poultry strains (Lu *et al.*, 2003a). Widespread bans on antimicrobials have led to renewed interest in the addition of moderate amounts of dietary fibre as a means to improve gut development, enzyme secretion and nutrient digestion in order to reduce enteric disorders (Kiarie *et al.*, 2013). As a substance, wood has been found to possess potent antibacterial properties that occur as a result of its hygroscopic properties and extractives (Milling *et al.*, 2005).

The most pronounced pathogens impacting poultry production are coccidia of the type *Eimeria,* bacteria such as *Escherichia coli* and numerous *Salmonella* and *Campylobacter* species. Several factors influence proliferation of these pathogens, such as the diet of the bird as well as the physical structure of the diet. Diets containing NSPs such as wheat can contribute to poor gut health since these dietary compounds are resistant to breakdown by the animal's enzymes. A viscous environment is then created that serves as an ideal condition for the proliferation of *Clostridium perfringens*, which occurs normally in the gut. Necrotic enteritis (NE), which results from *C. perfringens* proliferation, can cause some of the most severe intestinal lesions, with the bacterium's toxins causing necrotic lesions on the intestinal mucosa (Al-Sheikhly & Truscott, 1977). Necrotic enteritis can occur as an acute or subclinical disease. The acute form causes widespread mortality, whereas the subclinical form decreases digestion, absorption, weight gain and feed efficiency. Finely ground diets may increase mortalities associated with NE.

The most important gut diseases in poultry worldwide are NE and coccidiosis. Coccidiosis is the most important predisposing factor to NE. Coccidiosis is caused by *Eimeria* oocysts, a parasite that causes intestinal tissue damage, due to lysis of intestinal cells during the intracellular stage of the parasite's lifecycle (Williams, 2005). This increases mucogenesis and the mucin can be used as a substrate for *C. perfringens,* which then proliferates, and following which NE ensues (Williams, 2005).

Salmonellosis is a zoonotic disease that causes acute gastroenteritis in humans and is the cause of 93.8 million of cases of Salmonellosis annually worldwide, with some 155 000 acute cases resulting in deaths (Majowics *et al.*, 2010). Many mild cases are not reported, so the real figures could be much higher (Majowics *et al.*, 2010). Children, the elderly and immunocompromised persons are most at risk for Salmonellosis. In chickens, Salmonella infection occurs at a young age and presents as a chronic, asymptomatic infection. Maintaining hygienic conditions is important in preventing the introduction of Salmonella on the farm, or reducing its severity if it is present, however, these measures are not sufficient to prevent Salmonella outbreaks (European Food Safety Authority, 2004). Proper handling at the slaughterhouse may also decrease the contamination of carcasses (Kiarie *et al.*, 2013).

2.6 Effects of litter on gas emissions

A pioneering study found that body weight, feed conversion and housing conditions were adversely affected by increased ammonia emissions (Reece *et al.*, 1980). The ammonia is produced by poultry nitrogenous waste and bacterial metabolism. The main reason for the excess ammonia excretion is that protein levels in poultry feed are not perfectly balanced. For requirements of essential amino acids to be met, some amino acids are supplied in excess. Excess nitrogen, which is excreted in the form of uric acid, is rapidly converted to ammonia by hydrolysis, volatilisation, and mineralisation (Oenema *et al.*, 2001). The most prolific litter microbe responsible for the conversion of uric acid to ammonia is *Bacillus pasteurii* (Schefferle, 1965). Ammonia emission recommendations suggest that ammonia levels in poultry houses ought to be kept lower than 25 ppm. However, continuous exposure to 10 ppm may negatively impact bird health (Carlile, 1984).

Ammonia emissions are the most important when assessing gaseous emissions, apart from evaporated water, which was discussed at length. The drinker type has a significant influence on the ammonia emissions since different drinkers have a different frequency of usage. Elwinger & Svensson (1996) found that bell drinkers were associated with higher ammonia emissions than nipple drinkers ($P < 0.02$ and $P < 0.03$). The amount of litter used and the type of litter used (straw or wood shavings) did not influence ammonia emissions.

Microbial presence in litter and its effect on pH and ammonia emissions

Litter microbes contribute significant amounts to the total ammonia emissions in the house. The total amount of bacteria, Gram-positive, Gram-negative, *Staphylococcus* spp. and *E. coli,* were found to increase with increasing pH (Terzich *et al.*, 2000). If the litter is wet under cold or diseased conditions, fermentation of the litter increases. A rise in litter pH follows, which shifts the balance to alkaline (pH from 5 to 8, or higher). The high pH causes the uric acid to break down to form ammonia, which adversely affects air quality (Kleyn, 2013). The ammonia to ammonium ratio is determined by pH and as the pH increases, the degree of volatilisation increases (Moore *et al.,* 1996). Litter pH is generally alkaline and tends to increase in alkalinity as excreta accumulate over the production cycle (Garcês *et al.*, 2013).

High levels of ammonia also contribute to FPD, because the ammonia irritates the footpad epidermis and aids in proliferation of lesions. Opportunistic bacteria from the litter may cause secondary infection (Cengiz *et al.*, 2011).

2.7 Uses of poultry litter beyond the broiler house

Recycling possibility of litter

In many countries worldwide, litter is used for several cycles and it is allowed to build up. The SAPA Code of Practice (2012) recommended an all-in, all-out (AIAO) system which is widely practiced in South Africa. In countries such as Brazil, where litter is re-used, microbial loads of the litter become higher with each cycle, such that materials that are less favourable for bacterial growth are preferred (Garcia *et al.*, 2009).

Poultry litter in an American study by Lu *et al.* (2003a) was reported to have 10^9 aerobic bacteria per gram of litter. Only a fraction of the total bacteria was pathogenic, and a large proportion was similar to compost-forming organisms. The majority of aerobic bacteria consisted of Staphylococci, with enteric bacteria only 0.1% (enterococci) and 0.11% (gram-negative enterics), respectively. Reused litter may serve as an inoculant for the establishment of gut microflora for young chicks, which speeds up maturation of the gut microbiota (Torok *et al.*, 2009a).

Further uses

In terms of Act 36 of 1947, it is prohibited to feed poultry litter to livestock, except if it is registered as an animal feed. Nutritional consultants may not recommend the use of unregistered poultry litter. Farmers do, however, feed poultry litter under their own discretion, since it contains non-protein nitrogen and is relatively cheap. However, this must be done with caution, because the unregistered, unprocessed product can contain high levels of potentially toxic minerals, pathogenic bacteria, mycotoxins and parasites, as well as coccidiostats and antibiotics (Van Ryssen, 2001). Sundried poultry litter can be used as drought feeding for ruminants when mixed with molasses (Mavimbela *et al.*, 1997). It is also fed as part of ruminant rations as a winter feed, or as silage (Van Ryssen, 2001).

Poultry litter is also used successfully as fertilizer for crops (Chaudhry *et al.*, 2013; Garcês *et al.*, 2013). It contains a high degree of plant material and is of more value than manure from other farm animals. Poultry litter can replenish the nutrients lost from the soil due to crop production, and it can improve soil structure. With the rising costs of chemical fertilisers, poultry litter is becoming a viable alternative to crop farmers (Chaudhry *et al.*, 2013). However, due to nitrate pollution caused by run-off of poultry litter as crop fertiliser, other alternatives of utilising poultry litter have been explored.

Mushroom production relies on poultry manure as part of the growth medium for mushrooms. Approximately 30% of substrate for mushrooms consists of poultry litter (Richards, 2014). Poultry litter aids in improving ammonia levels needed for production of high quality mushrooms. Even when ammonia suppressants were used in deep-litter broiler production, poultry litter still provided adequate levels of ammonia to the mushrooms (González-Matute & Rinker, 2006).

Electricity from poultry litter is a modern way to harness biogas within organic products and utilise this to solve a two-way problem: disposing of the manure and alleviating the demand for electricity (Bon, 2015). In several countries, including South Africa, rebates from the government exist for customers who make use of renewable energy sources and can push excess electricity into the power grid.

2.8 Conclusion

It is clear that litter types and litter conditions have an impact on the performance, gut health, welfare, microbial load and disease incidence of broilers. Management of litter is thus important when choosing a bedding source, as is knowledge of physical characteristics of alternate litter sources. The ultimate impact of litter on poultry production is a multifactorial issue, where even small differences can make a large difference on tight profit margins.

CHAPTER 3

Materials and Methods

3.1 Housing

Six identical, fully environmentally controlled broiler houses in the location of Mpumalanga near Ogies at the Truter Group Tru-Cass Farm were utilised. The houses were controlled by the Big Dutchman climate control system and ViperTouch production computer. The houses were 123m x 15m in dimension, and were built in 2014. The trial was run in three AIAO cycles, one in October 2015, one in January and one in April/May 2016. Three bedding sources (treatments) were applied. There were two replicates of each treatment per cycle, thus 2 x 3 = 6 replicates per bedding source over three production cycles.

Three bedding sources were used:
- Bio-secure, fumigated virgin pine shavings (BS)
- Non-chemically treated pine shavings (PS)
- Sunflower hulls (SH)

Table 3.1. Allocation of bedding types across production cycles

House	Production cycle 1	Production cycle 2	Production cycle 3
1	Sunflower hulls	Pine shavings	Biosecure shavings
2	Sunflower hulls	Pine shavings	Biosecure shavings
3	Biosecure shavings	Sunflower hulls	Pine shavings
4	Biosecure shavings	Sunflower hulls	Pine shavings
5	Pine shavings	Biosecure shavings	Sunflower hulls
6	Pine shavings	Biosecure shavings	Sunflower hulls

Each bedding source was tested in each of the six houses, thus the house effect was accounted for in the study.

3.2 Broilers

All animal care procedures were done in accordance with the University of Pretoria's animal ethics committee guidelines (Project number EC 048-15).

Mixed-sex day-old chicks were received from the same hatchery (Midway Chix), and parent flock ages were recorded. Where possible, offspring from different parent flock ages were evenly distributed, such that over the three cycles, each bedding source treatment received a similar distribution in chicks from different parent flock ages. Ross 308 broilers were placed in the houses that were used for the trial. The stocking density was kept at approximately 42.8 kg/m^2 (23 birds/m^2) for each cycle. The total house capacity was 42 500 chicks. Broilers were slaughtered at 33 days of age.

General procedures

Broiler body weight, feed and water intake of birds were monitored daily per house, using automated flow-meters and Big Dutchman scales (Swing 20) in each house. Commercial feed conversion ratio was calculated at slaughter and broiler mortality was recorded at seven and 33 days, respectively. Weekly, 400 birds per house were weighed manually in the areas described in Figure 3.1, by cordoning off an area of 100 birds per location and weighing them in batches of ten birds in a crate with an Adam LBK 30 scale (d = 5 g, max = 30 kg).

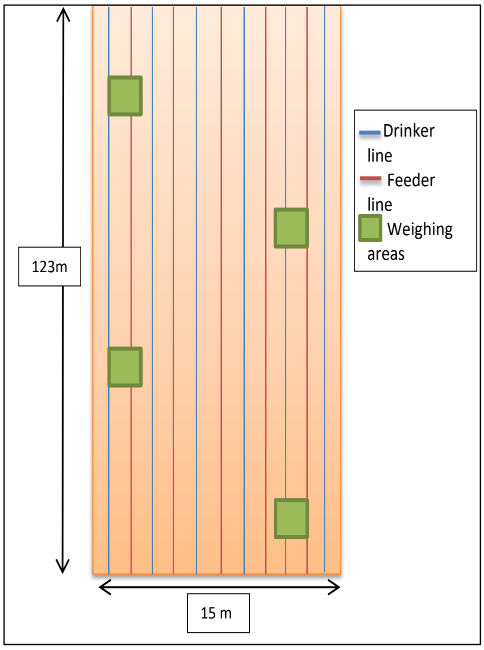

Figure 3.1. Broiler weighing locations

Gastrointestinal content and development: selection of birds

Proventriculus and gizzard content and development were assessed from 10 sacrificed birds per house at 21 and 31 days, respectively. The chosen birds seemed healthy and had a live weight close to the average weight for that age according to breed standard. The birds were selected for culling at the same locations used for weighing as seen in Figure 3.1. Two birds per location were selected from the back areas of each house, and three birds from the front areas of each house. After all birds in a house were selected, they were brought to a holding area in the front of the house, and culled. Selected birds were weighed using an Adam LBK 3 scale (where d = 0.5 g; max = 3 kg). Once culled, each bird was assigned a number from which further measurements would be done. Birds were culled by cervical dislocation by trained personnel. The breed standards for age and sex were used to select birds to be culled (Ross Broiler Performance Objectives, 2014) such that as far as possible equal ratios of male and female birds could be culled. All further measurements were calculated relative to the live weight of the selected broilers.

Gastrointestinal content and development: measurements

The digestive tract was excised from the proventriculus to the cloaca and mesentery and fat removed from the organs. Then, the gizzard and proventriculus were removed from the intestines at the proximal end of the duodenum. The full and empty proventriculus and gizzard were weighed using a laboratory scale (Mettler Toledo, PB303-SRS, d = 0.001 g, max = 310 g; Starck, 1999). The maximum length and width of the full gizzard as well as the width of the proventriculus sphincter were measured using vernier calipers (Raco tools, precision reaches 150 mm ± 0.1 mm; Amerah *et al.*, 2007). The gizzard was then opened and the content removed for moisture and fibre analysis. Dry matter was determined with AOAC method of analysis 934.01 and acid detergent fibre was determined with the Ankom Technology Method 8, filter bag technique for A2000 (See 3.8 Laboratory analysis methods). The gizzard contents were pooled in groups of two birds per house, thus five observations per house.

The intestinal length was measured using a flexible tape on a smooth surface to prevent inadvertent stretching (Amerah *et al.*, 2008). The lengths measured were from the proximal end of the duodenum to Merkel's diverticulum and from Merkel's diverticulum to the ileocaecal junction, as well as both caeca. Following measurement, the intestinal contents were squeezed out and the empty intestines were weighed.

3.3 Footpad dermatitis (FPD)

For the evaluation of FPD in a broiler house, scoring of the footpads is the most sensitive indicator of FPD and is preferred above examining hock or breast blisters. For this trial, footpads were scored in five classes in a similar fashion to the European system for scoring FPD in turkeys (Hocking *et al.*, 2008). See Table 3.2 below.

Table 3.2. Footpad dermatitis scoring method utilised during the trial

Class	Picture	Description
1		Footpad exhibits no external signs of FPD, footpad is soft to the touch, exhibiting normal colouration, with no redness.

2		Cracks may be seen between scales, swelling/redness evident, footpad has harder areas, and necrotic spots on single scales may be seen.
3		Hyperkeratosis of scales may be seen, necrosis of scales occurs, black necrotic area should not cover more than a quarter of the footpad.
4		Hyperkeratosis is seen around the lesion, lesion covers up to half of the footpad, marked swelling on footpad.
5		As for Score 4, but lesion covers more than half of the footpad, the scab from the lesion falls off easily and other matter may stick to the footpad due to oozing of the lesion.

The areas where FPD scoring was done in the houses were the same as for weighing, as shown in Figure 3.1. Scoring was conducted at 21 and 31 days of age.

3.4 Feed

A proximate analysis consisting of dry matter, ash, crude protein, acid detergent fibre and ether extract of the feed was done for each replication, according to AOAC procedures.

The following methods of analysis were used:

- Dry Matter Method 934.01;
- Ash Method 942.05;
- Crude protein Leco-Dumas Method 968.06;
- Acid detergent fibre-Ankom technology Method 8; and
- Ether Extract Method 920.39.

These methods are described in Section 3.8 (Laboratory analysis methods). As soon as feed was dispensed, a sample of the feed was taken from the central hopper of each house, and pooled together. A representative sample of the pre-starter, starter, grower, finisher and post-finisher was collected.

3.5 Bedding

General procedures

All litter materials were received from the same suppliers throughout the trial. The litter had a depth of 50 mm at placement of each production cycle. Bedding was evenly spread with a roller. Topdressing was done in areas where drinker nipples leaked or where caking scores reached 100%, and the wet litter was becoming a hazard to the broilers.

All litter types were subjected to a water-holding capacity test, a water-releasing capacity test, a bulk density test and a pH test (see detailed methods below) weekly. Analysis of moisture, ash, crude protein, crude fibre, acid detergent fibre (ADF) and ether extract at the commencement and conclusion of each cycle was done according to the AOAC procedures detailed for feed analysis above. These procedures are described in Section 3.8 (Laboratory analysis methods).

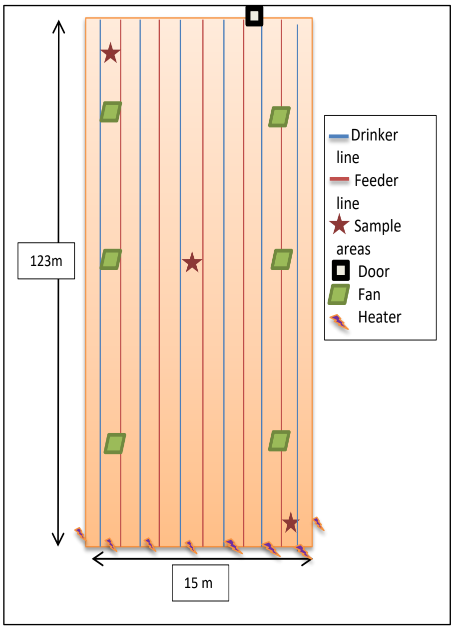

Figure 3.2. Litter sampling locations

Selection of litter samples

Samples were collected at three areas in the house, as indicated by Figure 3.2. All the litter in a 0.5m^2 area was removed, mixed well, and subsamples were taken for analysis. Weekly, the following was tested in the sample area: water-holding capacity, water-releasing capacity, pH, bulk density, litter moisture and crude protein.

Procedures

Bulk density was measured as the mass of as is litter that fits in a 1L beaker.

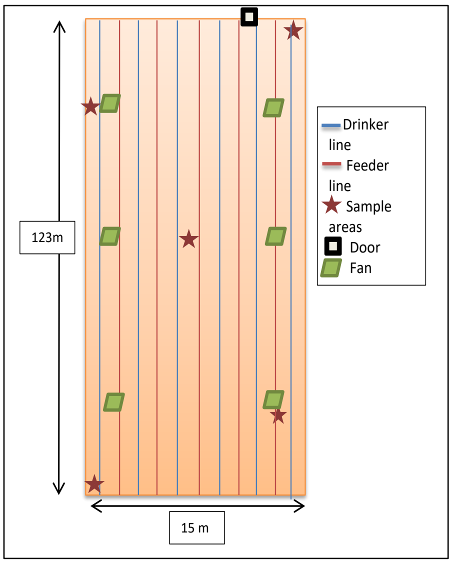

Figure 3.3. Litter caking score locations

Litter caking was measured with a percentage scoring system at 21 and 31 days, respectively. A 50 cm^2 frame was placed at five areas in the house as shown in Figure 3.3. The frame was flipped four times to form 1m^2. The percentage of caked litter in the square was estimated by evaluating the amount of caking in each quarter of the square. In each frame, scoring was done as follows: 0 = no caking in the square; 1 = ¼ of the square caked; 2 = ½ of the square caked; 3 = ¾ of square caked; and 4 = whole square caked. These values were then converted to percentages from fractions of four. Water-holding capacity was determined as follows: each litter sample was dried at 55°C until constant weight and 50g placed in a 500-mL beaker; the beaker was filled with water and left to stand for 30 minutes; excess water drained for 3 minutes through a 850µm sieve; and was then weighed again, where the percentage water absorbed was thereby calculated (Brake *et al.*, 1992; Garcês *et al.*, 2013).

Water-releasing capacity was determined by placing a 50g litter sample in a 15x20cm foil pan, filling it with water, and allowing it to stand for 30 minutes, after which it was drained through a 850 µm sieve for three minutes. The pan with its contents was weighed immediately after draining, after five hours and again after 24 hours.

Litter pH was determined by suspending a macerated 30g litter sample in 250mL distilled, deionised water, agitating for five minutes and measuring pH after half an hour with a Hanna pH meter HI8424 and electrode H1230 (Garcês *et al.*, 2013).

3.6 Litter beetle traps

Prior to the start of the trial, the houses utilised were fumigated to eliminate prior beetle infestation. No fumigation was applied in the open days between cycles while the trial was running, so all beetles that were to be counted arose from the trial. The litter beetle traps used were modified Arends Trap tubes, also used by Safrit & Axtell (1984), consisting of 23 cm PVC piping, with a diameter of 4 cm. Corrugated cardboard (30 cm x 20 cm and 5mm thick) was rolled up and placed inside the PVC pipe. The cardboard was replaced weekly, and the used cardboard sealed in a plastic bag, whereafter it was frozen for at least 48 hours. The beetles, pupae and worms were then shaken out and counted (Arends, 1987; Safrit & Axtell, 1984).

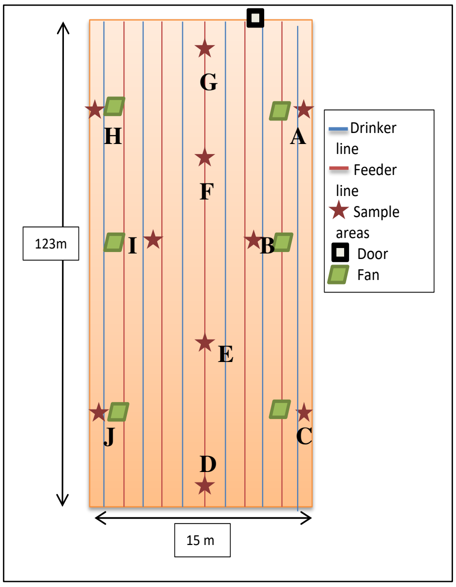

Figure 3.4. Beetle trap locations

The traps were placed at ten locations in the house (Figure 3.4), as recommended by Safrit & Axtell (1984) for routine monitoring. Positions in the house were chosen close to the wall (A, C, H, J), under the feeder lines (D, E, F, G) with position D under the motor and G under the feed hopper. There were also positions in the center of the house away from feeders and drinkers (B, I).

3.7 Bacterial monitoring

Boot swabs were taken for *Salmonella* monitoring at the beginning and end of each cycle. Boot swabs were obtained from and sent in to Deltamune laboratory in Centurion, Pretoria for *Salmonella* detection. Two paths were walked as indicated by Figure 3.5.

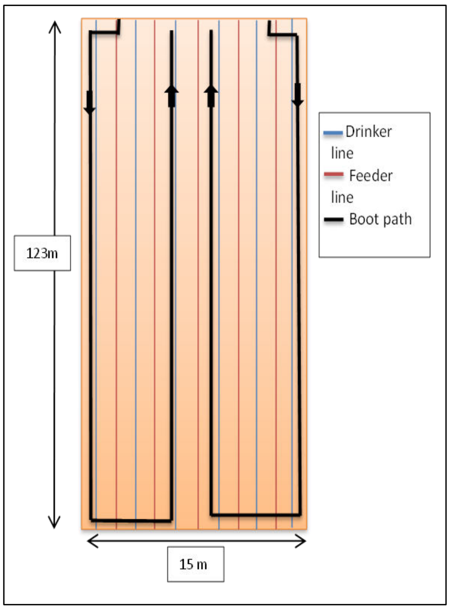

Figure 3.5. Boot sampling paths

3.8 Laboratory analysis methods

The detailed methods of each of the laboratory analyses performed for this trial are outlined below. All samples were analysed in duplicate.

Initial dry matter

The weight of a tin foil pan was recorded, after which 2g of wet sample was accurately weighed into the pan and the combined weight was recorded. Thereafter, the pan with the sample was placed in a 105°C oven for 24 hours. The sample was removed from the oven, placed in a desiccator for 30 minutes, and the dry weight was recorded. The remainder of the sample was dried at 55°C for 48 hours, milled to pass through a 1mm screen and bottled for further analysis. The mills used varied according to the type of samples milled. Litter samples were milled with the Retsch knife mill (Retsch GmbH, Model SM 100, Haan, Germany); feed samples were milled with a Retsch ultra-centrifugal mill (Retsch GmbH, Model ZM 200, Haan, Germany); and gizzard content samples were milled using a Cole-Palmer jar mill.

Dry Matter

Clean, dry crucibles were put in a 105°C oven for an hour, then 30 minutes in a desiccator to cool down, after which the weight of the empty crucibles were recorded. The 2g sample was then weighed into the crucible and put in a 105°C oven for 24 hours. After 24 hours, the crucibles with the samples were removed from the oven, placed in a desiccator for 30 minutes and weighed back. Dry matter was determined as follows: DM % = $\frac{mass\ of\ ovendried\ sample+mass\ of\ crucible}{mass\ of\ airdried\ sample+mass\ of\ crucible}$ X $\frac{100}{1}$

Ash

The dried sample from the dry matter analysis was used for this analysis. The crucible and sample was placed in an incinerating oven for two hours at 250°C, followed by four hours at 600°C. Following incineration, the oven was allowed to cool down for at least two hours before the samples were removed, placed in the desiccator and reweighed.

Crude Protein (Leco-Dumas method)

The sample amount of 0.2g was weighed in on a sample boat and inserted in the combustion tube of the Leco apparatus, where it was washed with oxygen to remove atmospheric contamination, before moving to the furnace for combustion. Any elemental nitrogen was converted to N_2 and NO_x gases. Following combustion, the gases were collected in a ballast tank and allowed to equilibrate before being passed through a reduction heater, which reduced the gases to N_2. Water and CO_2 were removed as the gases were passed through Anhydrone and Lecosorb, respectively. Only N_2 remained in the helium flow, which was analysed as it passed through a thermal conductivity cell. The final nitrogen content was displayed as percent nitrogen, which was multiplied by 6.25 to obtain percentage protein.

Ether Extract

A 2g sample was weighed into a tared Whatman filter paper No. 1. The filter paper was folded and placed in a thimble. Büchi beakers were removed from the 55°C oven, placed in the desiccator for a few minutes, and weighed. After weighing, the Büchi beakers were filled two-thirds with petroleum-ether with 40-60 °C boiling point. The thimbles were placed in the soxhlet extraction tube and the beakers sealed in. The sample was boiled in the ether for one hour and allowed to evaporate. The beakers with its' fat content were placed in the 55 °C oven overnight and reweighed.

$$\% \text{ Crude fat} = \frac{Mass\ crude\ fat}{Sample\ weight} \text{ x } 100$$

Acid detergent fibre

This analysis was done using the Ankom Technology Method 8, filter bag technique for A2000. An empty filter bag was weighed, and 0.45-0.55 g of sample was tipped into the filter bag and sealed. The bags were loaded into the bag suspender and placed into the apparatus, which was set for ADF and left for extraction and rinsing. The samples were removed and soaked in acetone for 3-5 minutes, left to air-dry and placed in a 55 °C oven overnight.

ADF % (as is) was calculated using the following equation:

$$\% \text{ ADF}_{\text{(as is)}} = \frac{(Dried\ bag\ after\ process - (Bag\ tare\ weight\ x\ Blank\ bag\ correction))}{Sample\ weight} \text{ x } 100$$

Statistical analysis

Data was statistically analysed as a completely randomised design with the GLM model of analysis using SAS software (Statistical Analysis System, 2013). Three treatments were applied with two replicates per treatment randomly assigned to each broiler house in each production cycle. The fixed effects were bedding source (treatment) and production cycle.

Several variables were assessed in this study. The litter physical characteristics examined included bulk density (BD), pH, water-holding capacity (WHC), and litter caking. Propensity to litter beetle infestation was analysed using the Chi-square method of analysis using SAS software (Statistical Analysis System, 2013). Proximate laboratory analysis of the litter materials included dry matter, crude protein, crude fibre, acid detergent fibre, ether extract and ash analyses. Broiler performance was measured by production parameters, namely: average daily gain (ADG); feed conversion ratio (FCR); production efficiency factor (PEF); slaughter weight, kilograms of broilers produced per m^2, mortalities and total feed consumed. Broiler gut development (weight and length) and footpad dermatitis scoring were also investigated.

CHAPTER 4
RESULTS

4.1 Feed composition

The broiler feed composition for each of the production cycles is outlined in Table 4.1.1 below. Broilers were fed in phases: pre-starter, starter, grower, finisher and post-finisher. The nutrient analysis did not include the finisher feed, as it had the same formulation as the post-finisher feed, but without medication.

Table 4.1.1. Comparison of nutrient values (on a DM basis) of broiler feed in the different production cycles

Feed	**Production Cycle**	**DM (%)**	**CP (%)**	**EE (%)**	**Ash (%)**	**ADF (%)**
Pre-starter	1	88.80	26.07	3.74	6.62	6.63
Pre-starter	2	89.06	25.04	3.26	6.51	6.20
Pre-starter	3	88.62	25.76	4.40	6.43	6.01
Starter	1	88.92	23.83	4.75	6.24	7.14
Starter	2	89.16	24.89	3.72	6.28	6.53
Starter	3	88.71	26.66	4.45	6.58	5.63
Grower	1	88.40	23.04	5.20	5.15	7.11
Grower	2	89.04	23.18	4.21	5.80	6.88
Grower	3	88.03	24.27	4.85	5.86	7.81
Post-finisher	1	88.21	21.77	5.47	5.13	7.74
Post-finisher	2	88.97	23.05	5.43	5.40	7.56
Post-finisher	3	87.74	22.25	5.27	4.28	6.50

4.2 Litter initial composition

The nutrient values of the bedding materials were analysed prior to chick placement in each of the production cycles, which is outlined in Table 4.2.1 below.

Table 4.2.1. Comparison of nutrient values of litter types prior to chick placement in the different production cycles

Litter	Production Cycle	DM initial (%)	DM (%)	CP (%)	EE (%)	Ash (%)	ADF (%)
Biosecure shavings	1	94.83	95.96	0.83	0.78	0.25	84.08
Biosecure shavings	2	94.16	94.88	0.97	0.53	1.59	83.60
Biosecure shavings	3	93.97	95.53	0.71	0.61	0.58	84.09
Pine shavings	1	93.59	95.91	0.98	1.77	0.46	81.31
Pine shavings	2	94.09	95.53	0.90	0.67	0.49	82.24
Pine shavings	3	94.37	96.17	0.75	0.73	0.49	83.22
Sunflower hulls	1	92.81	96.44	6.43	9.42	3.04	64.99
Sunflower hulls	2	91.86	96.17	6.34	8.85	3.18	63.44
Sunflower hulls	3	93.85	96.50	6.32	7.19	7.23	62.31

4.3 Physical characteristics of litter

4.3.1 Bulk density

Table 4.3.1.1. The initial bulk density (g/L) of the litter treatments at Day Zero

Litter type	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Mean across litter types**
Biosecure shavings	49.5^{a}	48.0^{a}	53.0^{a}	50.2^{x}
Pine shavings	133.5^{b}	123.0^{b}	110.5^{b}	122.3^{y}
Sunflower hulls	163.8^{b}	171.0^{c}	176.0^{c}	170.3^{z}
Mean across cycles	115.6	114.0	113.2	

^{a,b,c} Within columns, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{x,y,z} Across litter types, means with no common superscripts differ significantly ($P < 0.05$) from each other.

At Day Zero, the mean bulk density varied widely ($P < 0.0001$) between the litter treatments and BS had the lowest bulk density, followed by PS and SH had the highest bulk density. No difference ($P > 0.05$) was observed between cycles in the bulk density of litter at Day Zero of the production cycle. Within cycles across treatments, the bulk density from BS was lower ($P < 0.05$) than the other treatments during Cycle One. In cycles Two and Three, differences ($P < 0.05$) were noticed among all three treatments. The bulk density was the lowest for BS, the highest for SH and the bulk density of PS was intermediate to the other treatments. No differences ($P > 0.05$) were observed within treatments across cycles on any of the treatments.

Table 4.3.1.2. The bulk density (g/L) of the litter treatments at Day Seven

Litter type	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Mean across litter types**
Biosecure shavings	87.0^{a}	89.5^{a}	104.0^{a}	93.5^{x}
Pine shavings	141.0^{b}	142.5^{b}	146.0^{b}	143.2^{y}
Sunflower hulls	193.5^{c}	198.0^{c}	196.0^{c}	195.8^{z}
Mean across cycles	140.5	143.3	148.7	

^{a,b,c} Within columns, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{x,y,z} Across litter types, means with no common superscripts differ significantly ($P < 0.05$) from each other.

Across litter types, the mean bulk density at Day Seven varied widely ($P < 0.0001$) between the treatments. Biosecure shavings (BS) had the lowest bulk density, followed by PS and SH had the highest bulk density. No differences ($P > 0.05$) were observed between cycles in the bulk density of the litter at Day Seven of the production cycle. Within cycles across treatments in Cycle One, the bulk density between BS and SH differed significantly ($P < 0.0001$), and PS also differed ($P < 0.05$) from the other treatments. Similar trends were seen in cycles two and three where differences ($P < 0.05$)

were also noticed among treatments. Within treatments across cycles, no differences ($P > 0.05$) were observed in any of the treatments.

Table 4.3.1.3. The bulk density (g/L) of the litter treatments at Day 14

Litter type	Cycle 1	Cycle 2	Cycle 3	Mean across litter types
Biosecure shavings	233.0 ^{a,A}	130.5 ^{a,B}	180.5 ^{a,C}	181.3 ^{x}
Pine shavings	223.5 ^{a,A}	232.0 ^{b,A}	222.0 ^{ab,A}	225.8 ^{y}
Sunflower hulls	250.0 ^{a,A}	213.0 ^{b,A}	242.0 ^{b,A}	235.0 ^{y}
Mean across cycles	235.5 ^{X}	191.8 ^{Y}	214.8 ^{X}	

^{a,b} Within columns, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{A,B,C} Within rows, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{x,y} Across litter types, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{X,Y} Across cycles, means with no common superscripts differ significantly ($P < 0.05$) from each other.

The mean bulk density across litter types indicated that BS had a lower ($P < 0.05$) bulk density when compared to other treatments. The mean bulk density across cycles indicated that litter in Cycle Two had a lower bulk density ($P < 0.05$) when compared to the other cycles. Within cycles across treatments, no difference ($P > 0.05$) was observed across treatments in Cycle One, whereas in Cycle Two, BS had a lower bulk density ($P < 0.05$) when compared to the other treatments. In Cycle Three, the bulk density of BS and SH differed significantly ($P < 0.05$). Within treatments across cycles, differences ($P < 0.05$) were only seen in the bulk density of BS in all three cycles with the lowest bulk density in Cycle Two, and the highest bulk density in Cycle One.

Table 4.3.1.4. The bulk density (g/L) of the litter treatments at Day 21

Litter type	Cycle 1	Cycle 2	Cycle 3	Mean across litter types
Biosecure shavings	296.0 ^{a}	268.0 ^{a}	339.5 ^{a}	301.2
Pine shavings	318.5 ^{a}	343.0 ^{b}	312.0 ^{a}	324.5
Sunflower hulls	329.0 ^{a}	324.5 ^{ab}	326.0 ^{a}	326.5
Mean across cycles	314.5	311.8	325.8	

$^{a,\ b}$ Within columns, means with no common superscripts differ significantly ($P < 0.05$) from each other.

At Day 21, the mean bulk density across litter types indicated no differences ($P > 0.05$) between treatments or cycles. Within cycles across treatments, no differences ($P > 0.05$) were observed across treatments in cycles One and Three. In Cycle Two, the bulk density of BS and PS differed ($P < 0.05$) from each other. No differences ($P > 0.05$) were observed within treatments across cycles.

Table 4.3.1.5. The bulk density (g/L) of the litter treatments at Day 31

Litter type	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Mean across litter types**
Biosecure shavings	383.0	362.0	381.0	375.3
Pine shavings	374.0	411.0	425.0	403.3
Sunflower hulls	368.0	366.5	345.0	359.8
Mean across cycles	375.0	379.8	383.7	

The bulk density of litter at Day 31 of the production cycle indicated no differences ($P > 0.05$) across treatments or across cycles.

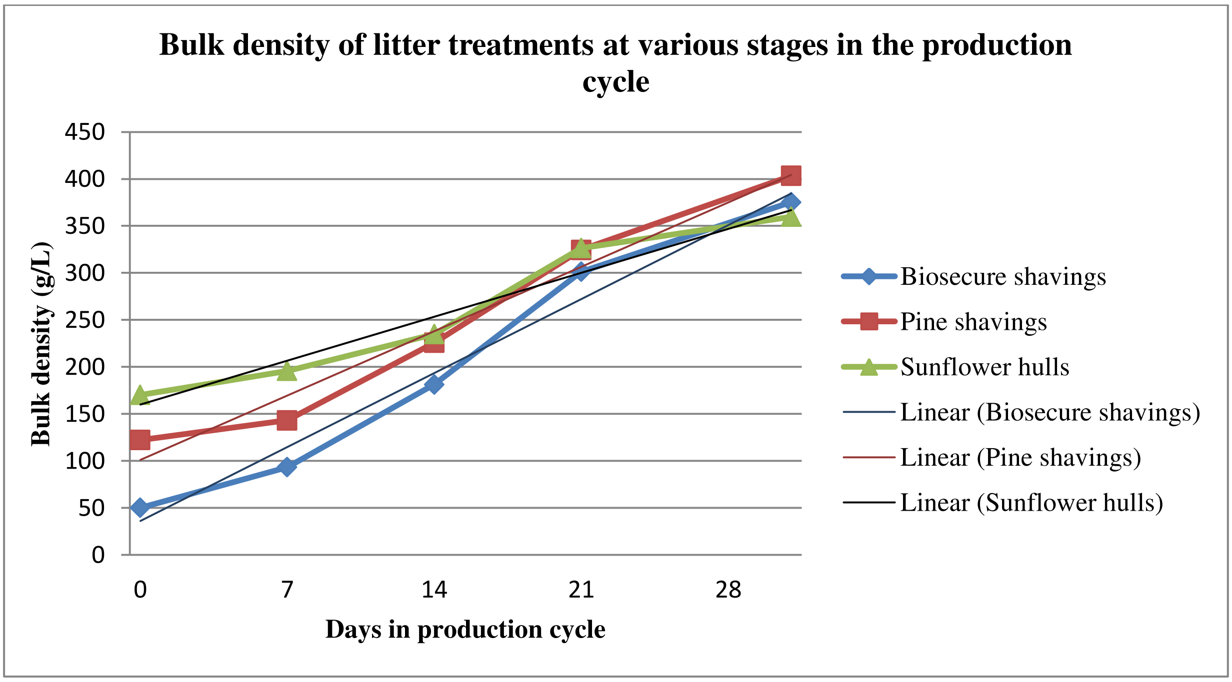

Figure 4.3.1. Comparison of bulk density of litter treatments at various stages in the production cycle

Table 4.3.1.6. Comparison of lines of best fit, R^2, and P-values of the different litter types

Litter type	**Equation**	R^2	**P-value**
Biosecure shavings	$y = 11.54x + 29.48$	0.9805	0.0012
Pine shavings	$y = 10.06x + 94.96$	0.9784	0.0014
Sunflower hulls	$y = 6.85x + 156.07$	0.9561	0.0040

The litter bulk density followed a linear pattern. The SH had the smallest increase in bulk density and BS and PS increased at fairly similar rates.

4.3.2 Litter pH

Due to a faulty pH meter, initial pH measurements could not be compared.

Table 4.3.2.1. Litter pH of the treatments at Day Seven

Litter type	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Mean across litter types**
Biosecure shavings	$6.04^{a,A}$	$6.42^{a,B}$	$5.88^{a,A}$	6.11^{x}
Pine shavings	$5.85^{b,A}$	$6.09^{b,B}$	$6.03^{a,AB}$	5.99^{y}
Sunflower hulls	$5.92^{ab,A}$	$6.28^{a,B}$	$6.02^{a,A}$	6.07^{xy}
Mean across cycles	5.94^{X}	6.26^{Y}	5.98^{X}	

^{a,b} Within columns, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{A,B} Within rows, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{x,y} Across litter types, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{X,Y} Across cycles, means with no common superscripts differ significantly ($P < 0.05$) from each other.

At Day Seven the mean pH across litter types indicated that PS had the lowest pH ($P < 0.05$), BS had the highest pH ($P < 0.05$), and SH had a pH intermediate to these litter types ($P > 0.05$). The mean pH across cycles indicated that litter in Cycle Two had a higher pH ($P < 0.05$) than in the other cycles. Across litter types within Cycle One, PS had the lowest pH ($P < 0.05$), BS had the highest pH ($P < 0.05$) and SH had a pH intermediate to these litter types ($P > 0.05$). Across litter types within Cycle Two, PS had a lower pH ($P < 0.05$) than the other treatments. Across litter types within Cycle Three, no difference ($P > 0.05$) was found across treatments. Across cycles within treatments, BS and SH had a higher pH ($P < 0.05$) in Cycle Two when compared to the other cycles. The PS had the lowest pH ($P < 0.05$) in Cycle One, the highest pH ($P < 0.05$) in Cycle Two and Cycle Three had a pH intermediate to the other cycles ($P > 0.05$).

Table 4.3.2.2. Litter pH of the treatments at Day 14

Litter type	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Mean across litter types**
Biosecure shavings	$6.52^{a,A}$	$7.00^{ab,A}$	$6.98^{a,A}$	6.83^{xy}
Pine shavings	$6.44^{a,A}$	$7.77^{a,B}$	$7.22^{a,B}$	7.14^{x}
Sunflower hulls	$6.17^{a,A}$	$6.77^{b,A}$	$6.72^{a,A}$	6.55^{y}
Mean across cycles	6.38^{X}	7.18^{Y}	6.97^{Y}	

^{a,b} Within columns, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{A,B} Within rows, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{x,y} Across litter types, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{X,Y} Across cycles, means with no common superscripts differ significantly ($P < 0.05$) from each other.

At Day 14, the mean pH across litter types indicated that the lowest pH ($P < 0.05$) was found on SH, the highest pH ($P < 0.05$) was found on PS, and BS had a pH intermediate to these litter types ($P > 0.05$). The mean pH across cycles indicated that litter in Cycle One had a lower pH ($P < 0.05$) than in the other cycles. Across litter types within cycles One and Three no difference ($P > 0.05$) was found across treatments. In Cycle Two, SH had the lowest pH ($P < 0.05$), PS had the highest pH ($P < 0.05$) and BS had a pH intermediate to these litter types ($P > 0.05$). Across cycles within treatments, no differences ($P > 0.05$) in pH were found across cycles between BS and SH. The pH in Cycle One was lower ($P < 0.05$) than in the other cycles on PS.

Table 4.3.2.3. Litter pH of the treatments at Day 21

Litter type	Cycle 1	Cycle 2	Cycle 3	Mean across litter types
Biosecure shavings	7.62^{A}	8.50^{A}	8.19^{A}	8.10
Pine shavings	6.92^{A}	8.42^{B}	8.49^{B}	7.94
Sunflower hulls	7.01^{A}	8.38^{B}	7.72^{AB}	7.70
Mean across cycles	7.18^{X}	8.43^{Y}	8.13^{Y}	

^{A,B} Within rows, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{X,Y} Across cycles, means with no common superscripts differ significantly ($P < 0.05$) from each other.

At Day 21, the mean pH across litter types indicated no difference ($P > 0.05$) between the various litter types. The mean pH across cycles indicated that litter in Cycle One had a lower pH ($P < 0.05$) than in the other cycles. Across litter types within cycles, no difference ($P > 0.05$) was found across treatments. Across cycles within treatments for BS, no difference ($P > 0.05$) was found across cycles, but for PS, the pH in Cycle One was lower ($P < 0.05$) than in the other cycles. For SH, Cycle One had the lowest pH ($P < 0.05$), Cycle Two had the highest pH ($P < 0.05$), and Cycle Three had a pH intermediate to the other cycles ($P > 0.05$).

Table 4.3.2.4. Litter pH of the treatments at Day 31

Litter type	Cycle 1	Cycle 2	Cycle 3	Mean across litter types
Biosecure shavings	$8.58^{a,AB}$	$7.64^{ab,A}$	$8.98^{a,B}$	8.40
Pine shavings	$8.47^{a,A}$	$8.53^{a,A}$	$8.43^{a,A}$	8.47
Sunflower hulls	$8.76^{a,A}$	$7.16^{b,B}$	$8.84^{a,A}$	8.25
Mean across cycles	8.60^{X}	7.77^{Y}	8.75^{X}	

^{a,b} Within columns, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{A,B} Within rows, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{X,Y} Across cycles, means with no common superscripts differ significantly ($P < 0.05$) from each other

At Day 31, the mean pH across litter types indicated no difference ($P > 0.05$) between the various litter types. The mean pH across cycles indicated that litter in Cycle Two had a lower pH ($P < 0.05$) than in the other cycles. Across litter types within cycles no differences ($P > 0.05$) were found across treatments, except in Cycle Two, where SH had the lowest pH ($P < 0.05$), PS had the highest pH ($P < 0.05$) and BS had a pH intermediate to these litter types ($P > 0.05$). Across cycles within treatments for PS, no difference ($P > 0.05$) was found across cycles, but for SH, the pH in Cycle Two was lower ($P < 0.05$) than in the other cycles. For BS, Cycle Two had the lowest pH ($P < 0.05$), Cycle Three had the highest pH ($P < 0.05$), and Cycle One had a pH intermediate to the other cycles ($P > 0.05$).

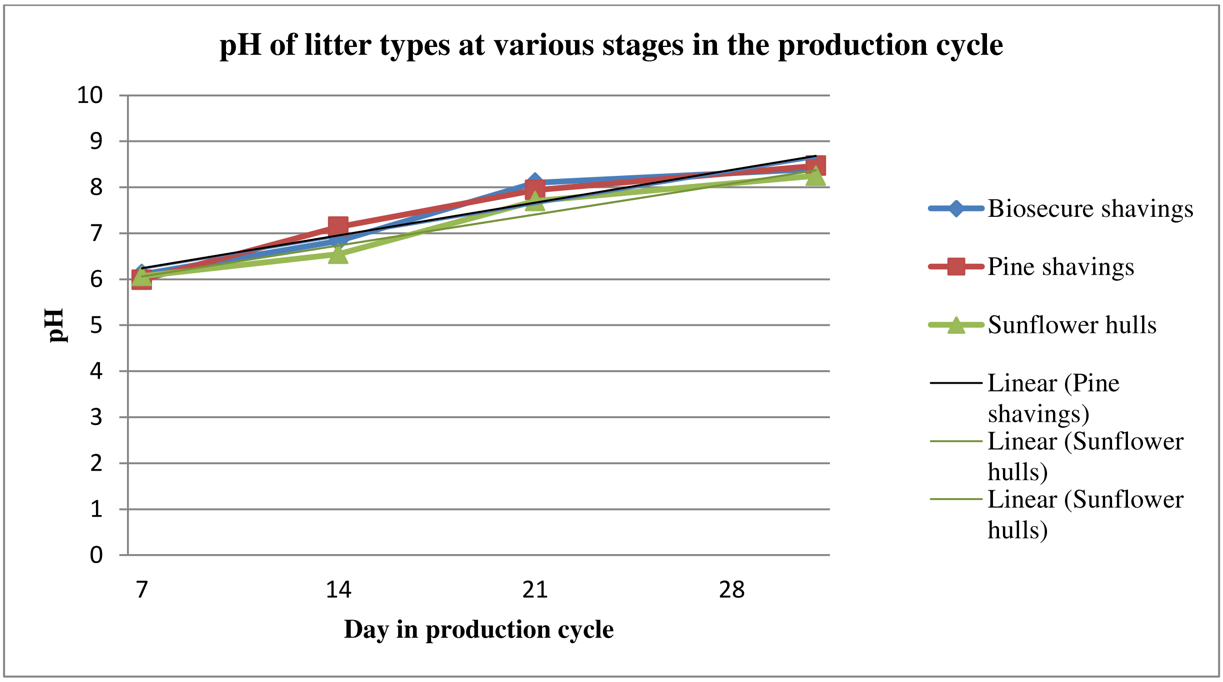

Figure 4.3.2. Comparison of pH of litter types at various stages in the production cycle

Table 4.3.2.5. Comparison of lines of best fit, R^2, and P-values of the different litter types

Litter type	**Equation**	**R^2**	**P-value**
Biosecure shavings	$y = 0.10x + 5.53$	0.9142	0.0439
Pine shavings	$y = 0.10x + 5.52$	0.9374	0.0318
Sunflower hulls	$y = 0.096x + 5.38$	0.9560	0.0223

Litter pH increased at similar linear rates as the production cycles progressed.

4.3.3 Litter caking

Table 4.3.3.1. Litter caking mean (%) of the treatments at Day 21

Litter type	Cycle 1	Cycle 2	Cycle 3	Mean across litter types
Biosecure shavings	$40.00^{a,A}$	$82.50^{a,B}$	$57.53^{a,A}$	60.01^{x}
Pine shavings	$11.88^{b,A}$	$83.13^{a,B}$	$43.13^{a,C}$	46.04^{y}
Sunflower hulls	$37.50^{a,A}$	$93.75^{a,B}$	$42.50^{a,A}$	57.92^{xy}
Mean across cycles	29.79^{X}	86.46^{Y}	47.72^{Z}	

^{a,b} Within columns, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{A,B,C} Within rows, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{x,y} Across litter types, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{X,Y,Z} Across cycles, means with no common superscripts differ significantly ($P < 0.05$) from each other.

At Day 21, the mean litter caking percentage across litter types indicated that BS caked more ($P < 0.05$) when compared with PS and SH had an intermediate caking percentage ($P > 0.05$). The mean litter caking percentage across cycles indicated that Cycle Two had a higher ($P < 0.0001$) caking score when compared with the other cycles, and that cycles One and Three also differed significantly ($P < 0.05$) from one another. Within cycles across treatments, PS had less caked litter ($P < 0.05$) in Cycle One when compared to the other cycles. No difference ($P > 0.05$) was observed across treatments in Cycle Two and Three. Within treatments across cycles, all three treatments had significantly higher ($P < 0.05$) caking percentages in Cycle Two, when compared to the other cycles. On PS, there were large ($P < 0.05$) differences between the cycles. No significant ($P > 0.05$) difference was observed on BS and SH between Cycle One and Three.

Table 4.3.3.2. Litter caking mean (%) of the treatments at Day 31

Litter type	Cycle 1	Cycle 2	Cycle 3	Mean across litter types
Biosecure Shavings	$74.69^{a,A}$	$94.38^{a,B}$	$93.75^{a,B}$	87.60
Pine Shavings	$66.88^{a,A}$	$94.38^{a,B}$	$97.19^{a,B}$	86.15
Sunflower hulls	$70.00^{a,A}$	$97.50^{a,B}$	$78.13^{b,A}$	81.88
Mean across cycles	70.52^{X}	95.42^{Y}	89.69^{Y}	

^{a,b} Within columns, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{A,B} Within rows, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{X,Y} Across cycles, means with no common superscripts differ significantly ($P < 0.05$) from each other

At Day 31 no difference ($P > 0.05$) was observed in the mean caking percentage across litter types. The mean litter caking percentage across cycles indicated that Cycle One had less caking ($P < 0.05$) when compared to the other cycles. Within cycles across treatments, no difference ($P > 0.05$) was observed in the caking percentage across litter types, except in Cycle Three, where the caking percentage on SH was significantly lower ($P < 0.05$) when compared to the other treatments. Within treatments across cycles, for both BS and PS, a similar trait was seen where the treatment in Cycle One had a lower ($P < 0.05$) caking percentage when compared to the other treatments. The SH treatment showed that only Cycle Two had a higher ($P < 0.05$) litter caking percentage when compared to the other cycles.

4.3.4 Water-holding capacity

Table 4.3.4.1. Water-holding capacity (g H_2O/g) of litter at day 0

Litter type	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Mean across litter types**
Biosecure shavings	$2.90^{a,A}$	$3.48^{a,B}$	$3.02^{a,A}$	3.13^{x}
Pine shavings	$3.17^{a,A}$	$3.58^{a,B}$	$3.10^{a,A}$	3.28^{x}
Sunflower hulls	$1.64^{b,A}$	$1.76^{b,A}$	$1.60^{b,A}$	1.67^{y}
Mean across cycles	2.57^{X}	2.94^{Y}	2.57^{X}	

^{a,b} Within columns, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{A,B} Within rows, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{x,y} Across litter types, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{X,Y} Across cycles, means with no common superscripts differ significantly ($P < 0.05$) from each other.

Sunflower hulls had a lower ($P < 0.0001$) water-holding capacity (WHC) than the other treatments when the mean WHC across litter types was investigated at Day Zero of the production cycle. The mean WHC of litter across cycles indicated that litter in Cycle Two had higher ($P < 0.05$) WHC than in the other cycles. Within cycles across treatments in each cycle, SH had a lower ($P < 0.05$) WHC than did the other treatments. Within treatments across cycles in both PS and BS, the WHC was higher ($P < 0.05$) in Cycle Two when compared with the other cycles. No difference ($P > 0.05$) was reported in the WHC of SH across the cycles.

Table 4.3.4.2. Water-holding capacity (g H_2O/g) of litter at Day Seven

Litter type	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Mean across litter types**
Biosecure shavings	2.67^{a}	2.62^{a}	2.46^{a}	2.58^{x}
Pine shavings	2.58^{a}	2.74^{a}	2.63^{a}	2.65^{x}
Sunflower hulls	1.69^{b}	1.67^{b}	1.69^{b}	1.68^{y}
Mean across cycles	2.31	2.34	2.26	

^{a,b} Within columns, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{x,y} Across litter types, means with no common superscripts differ significantly ($P < 0.05$) from each other

Sunflower hulls had a lower ($P < 0.0001$) WHC than the other treatments when the mean WHC across litter types was investigated at Day Seven of the production cycle. The mean WHC of litter across cycles indicated no difference ($P < 0.05$) across cycles. Within cycles across treatments in each cycle, SH had a lower ($P < 0.05$) WHC than the other treatments. Within treatments across cycles no difference ($P < 0.05$) was observed across cycles.

Table 4.3.4.3. Water-holding capacity (g H_2O/g) of litter at Day 14

Litter type	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Mean across litter types**
Biosecure shavings	$1.95^{a,A}$	$2.16^{a,AB}$	$2.31^{a,B}$	2.14^{x}
Pine shavings	$2.01^{a,A}$	$2.17^{a,A}$	$2.19^{a,A}$	2.12^{x}
Sunflower hulls	$1.95^{a,A}$	$1.49^{b,B}$	$1.79^{b,AB}$	1.74^{y}
Mean across cycles	1.97	1.94	2.10	

^{a,b} Within columns, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{A,B} Within rows, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{x,y} Across litter types, means with no common superscripts differ significantly ($P < 0.05$) from each other.

At Day 14, sunflower hulls had a lower ($P < 0.05$) WHC than the other treatments when the mean WHC across litter types was investigated. The mean WHC of litter across cycles indicated no difference ($P > 0.05$) across cycles. Within cycles across treatments, no difference ($P > 0.05$) was observed among treatments in Cycle One, however, in Cycle Two and Three, the WHC of SH was lower ($P < 0.05$). Within treatments across cycles, BS had a higher ($P < 0.05$) WHC in Cycle Three than in the other cycles. No difference ($P > 0.05$) was observed in PS and in SH, WHC was the highest ($P < 0.05$) in Cycle One.

Table 4.3.4.4. Water-holding capacity (g H_2O/g) of litter at Day 31

Litter type	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Mean across litter types**
Biosecure shavings	2.15^{A}	1.47^{A}	2.09^{A}	1.90
Pine shavings	2.53^{A}	1.37^{B}	1.77^{B}	1.89
Sunflower hulls	2.36^{A}	1.71^{A}	1.64^{A}	1.90
Mean across cycles	2.35^{X}	1.51^{Y}	1.83^{Y}	

^{A,B} Within rows, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{X,Y} Across cycles, means with no common superscripts differ significantly ($P < 0.05$) from each other.

At Day 31 there was no difference ($P > 0.05$) observed between the treatments when the mean WHC across litter types was investigated. The mean WHC of litter across cycles indicated that litter had a higher ($P < 0.05$) WHC in Cycle One. Within cycles across treatments, no difference ($P > 0.05$) was observed between any of the treatments. Within treatments across cycles, higher ($P < 0.05$) WHC was only observed in PS in Cycle One. No other differences ($P > 0.05$) were observed.

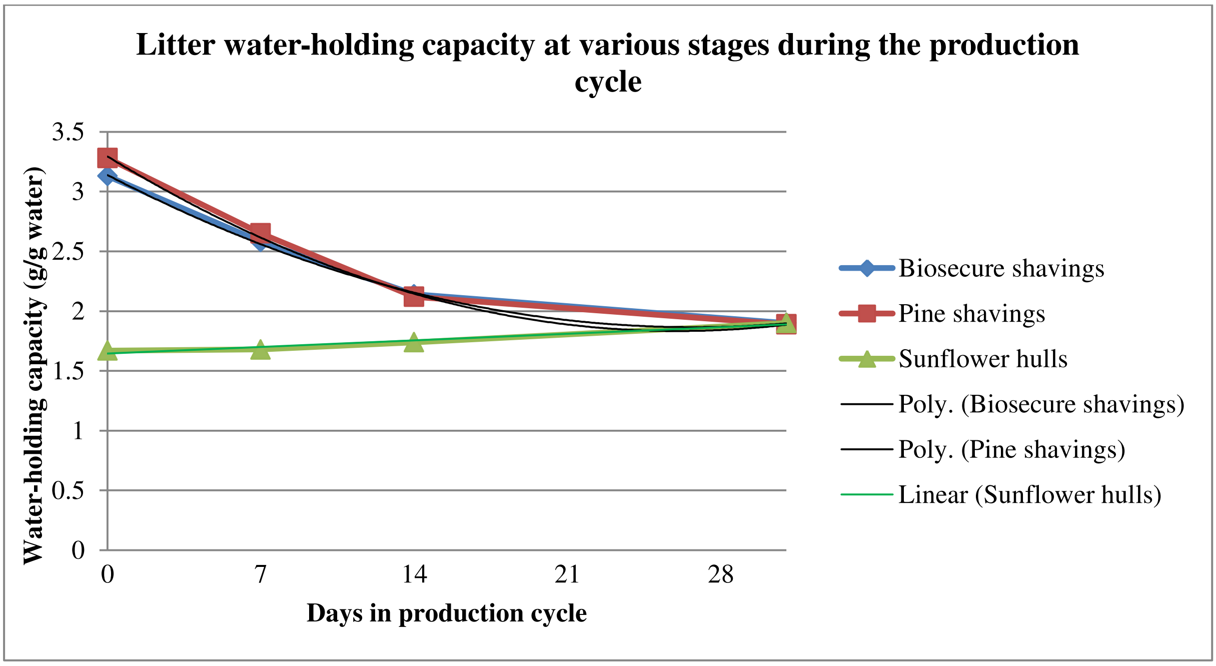

Figure 4.3.4 - Litter water-holding capacity at various stages during the production cycle

Table 4.3.4.5. Comparison of lines of best fit, R^2, and P-values of the different litter types

Litter type	**Equation**	**R^2**	**P-value**
Biosecure shavings	$y = 0.002x^2 - 0.11x + 3.23$	0.9999	0.0052
Pine shavings	$y = 0.002x^2 - 0.12x + 3.41$	0.9999	0.0083
Sunflower hulls	$y = 0.008x + 1.64$	0.9699	0.0151

The mean WHC of litter across a production cycle in depicted in the above graph. Both BS and PS have very similar quadratic equations, but SH follow a linear model. The WHC of BS and PS declined steadily over the production cycles, but SH actually increased in WHC. The litter types converged towards similar WHC at the end of the production cycle.

4.3.5 Water-releasing capacity of litter

Table 4.3.5.1. Water-releasing capacity (%) of litter after five hours at Day Zero

Litter type	Cycle 1	Cycle 2	Cycle 3	Mean across litter types
Biosecure shavings	$5.50^{a,A}$	$3.60^{a,B}$	$5.84^{a,A}$	4.98^{x}
Pine shavings	$4.44^{a,A}$	$3.02^{a,B}$	$4.40^{b,A}$	3.95^{y}
Sunflower hulls	$6.72^{b,A}$	$3.20^{a,B}$	$5.69^{ab,AB}$	5.21^{x}
Mean across cycles	5.55^{X}	3.28^{Y}	5.31^{X}	

^{a,b} Within columns, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{A,B} Within rows, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{x,y} Across litter types, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{X,Y} Across cycles, means with no common superscripts differ significantly ($P < 0.05$) from each other.

After five hours at Day Zero, pine shavings had a lower ($P < 0.05$) WRC compared to the other treatments when the mean WRC across litter types was investigated. The mean WRC of litter across cycles indicated that litter had a lower ($P < 0.0001$) WRC in Cycle Two as compared to the other cycles. Within cycles across treatments, SH had a higher ($P < 0.05$) WRC than the other treatments in Cycle One, whereas there were no differences ($P > 0.05$) among treatments in Cycle Two. In Cycle Three, BS had a higher WRC ($P < 0.05$) when compared to PS and SH had an intermediate WRC to the other treatments. Within treatments across cycles, WRC of all treatments was lower ($P < 0.05$) during Cycle Two than it was in the other cycles.

Table 4.3.5.2. Water-releasing capacity (%) of litter after 24 hours at Day Zero

Litter type	Cycle 1	Cycle 2	Cycle 3	Mean across litter types
Biosecure shavings	$13.67^{a,A}$	$18.22^{a,B}$	$25.87^{a,C}$	19.25^{x}
Pine shavings	$8.83^{b,A}$	$14.60^{ab,B}$	$20.32^{b,C}$	14.58^{y}
Sunflower hulls	$10.28^{ab,A}$	$14.02^{a,A}$	$20.64^{b,B}$	14.98^{y}
Mean across cycles	10.93^{X}	15.61^{Y}	22.28^{Z}	

^{a,b} Within columns, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{A,B,C} Within rows, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{x,y} Across litter types, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{X,Y,Z} Across cycles, means with no common superscripts differ significantly ($P < 0.05$) from each other.

After 24 hours at Day Zero, biosecure shavings had a higher ($P < 0.05$) WRC compared to the other treatments when the mean WRC across litter types was investigated. The mean WRC across cycles indicated that litter differed ($P < 0.05$) in each cycle, with large differences in Cycle Three ($P < 0.0001$). Across treatments within cycles in Cycle One, BS had a higher WRC ($P < 0.05$) when compared to PS. The SH had an intermediate WRC ($P > 0.05$) to the other treatments. In Cycle Two BS had a higher WRC ($P < 0.05$) when compared to SH and PS had an intermediate WRC ($P > 0.05$) to the other treatments. In Cycle Three, BS had a higher WRC when compared to the other treatments. Within litter types across cycles, BS and PS had differing WRC in each cycle ($P < 0.05$) and SH had a higher WRC ($P < 0.05$) in Cycle Three.

Table 4.3.5.3. Water-releasing capacity (%) of litter after five hours at Day Seven

Litter type	Cycle 1	Cycle 2	Cycle 3	**Mean across litter types**
Biosecure shavings	17.24 ^{ab,A}	4.38 ^{a,B}	4.79 ^{a,B}	8.80
Pine shavings	14.83 ^{a,A}	4.46 ^{a,B}	4.80 ^{a,B}	8.03
Sunflower hulls	18.65 ^{b,A}	4.83 ^{a,B}	4.58 ^{a,B}	9.35
Mean across cycles	16.91 ^{X}	4.56 ^{Y}	4.72 ^{Y}	

^{a,b} Within columns, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{A,B} Within rows, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{X,Y} Across cycles, means with no common superscripts differ significantly ($P < 0.05$) from each other.

After five hours at Day Seven no difference ($P > 0.05$) was found between treatments when the mean WRC across litter types was investigated. The mean WRC of litter across cycles indicated that litter had a higher ($P < 0.0001$) WRC in Cycle One, compared to the other cycles. Within cycles across treatments, WRC of PS and SH differed ($P < 0.05$) from each other in Cycle One, but no other differences ($P > 0.05$) were observed between treatments in the other cycles. Within treatments across cycles, all treatments in Cycle One had a ($P < 0.0001$) higher WRC when compared with the other cycles, but no other differences ($P > 0.05$) were observed.

Table 4.3.5.4. Water-releasing capacity (%) of litter after 24 hours at Day Seven

Litter type	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Mean across litter types**
Biosecure shavings	39.85 ^{ab,A}	17.16 ^{a,B}	21.35 ^{a,B}	26.12
Pine shavings	34.08 ^{a,A}	17.52 ^{a,B}	20.95 ^{a,B}	24.17
Sunflower hulls	42.33 ^{b,A}	17.96 ^{a,B}	17.96 ^{a,B}	26.08
Mean across cycles	38.74 ^{X}	17.55 ^{Y}	20.08 ^{Y}	

^{a,b} Within columns, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{A,B} Within rows, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{X,Y} Across cycles, means with no common superscripts differ significantly ($P < 0.05$) from each other

After 24 hours at Day Seven the mean WRC across treatments indicated no difference ($P < 0.05$) across litter types. The mean WRC across cycles indicated that litter in Cycle One was higher (P <

0.0001) than the other cycles. Across litter types within cycles, no differences were found across treatments in either Cycle Two or Cycle Three. However, in Cycle One, SH had a higher WRC ($P < 0.05$) when compared to PS, and SH had an intermediate WRC ($P > 0.05$) to the other treatments. In the treatments across cycles, all the litter types showed an increased WRC in Cycle One ($P < 0.05$) when compared with the later cycles.

Table 4.3.5.5. Water-releasing capacity (%) of litter after five hours at Day 14

Litter type	Cycle 1	Cycle 2	Cycle 3	Mean across litter types
Biosecure shavings	$8.37^{a,A}$	$2.83^{a,B}$	$2.58^{a,B}$	4.59^{x}
Pine shavings	$6.35^{a,A}$	$2.78^{a,B}$	$3.03^{a,B}$	4.05^{x}
Sunflower hulls	$14.32^{b,A}$	$3.20^{a,B}$	$3.17^{a,B}$	6.90^{y}
Mean across cycles	9.68^{X}	2.93^{Y}	2.92^{Y}	

^{a,b} Within columns, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{A,B} Within rows, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{x,y} Across litter types, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{X,Y} Across cycles, means with no common superscripts differ significantly ($P < 0.05$) from each other.

After five hours at Day 14, the mean WRC across litter types was compared and SH had a higher WRC ($P < 0.05$) in relation to the other treatments. The mean WRC of litter across cycles indicated that litter had a higher ($P < 0.0001$) WRC in Cycle One, compared to the other cycles. Within cycles across treatments, the only difference was found on SH in Cycle One, which had a higher ($P < 0.05$) WRC. Within treatments across cycles, all treatments in Cycle One had a significantly ($P < 0.05$) higher WRC when compared with the other cycles, but SH in Cycle One differed highly significantly ($P < 0.0001$).

Table 4.3.5.6. Water-releasing capacity (%) of litter after 24 hours at Day 14

Litter type	Cycle 1	Cycle 2	Cycle 3	Mean across litter types
Biosecure shavings	$32.86^{a,A}$	$16.50^{a,B}$	$11.66^{a,B}$	20.34^{x}
Pine shavings	$25.18^{b,A}$	$16.18^{a,B}$	$10.70^{a,B}$	17.35^{x}
Sunflower hulls	$47.64^{c,A}$	$17.18^{a,B}$	$13.03^{a,B}$	25.95^{y}
Mean across cycles	35.22^{X}	16.62^{Y}	11.80^{Z}	

^{a,b,c} Within columns, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{A,B} Within rows, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{x,y} Across litter types, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{X,Y,Z} Across cycles, means with no common superscripts differ significantly ($P < 0.05$) from each other.

After 24 hours at Day 14, the mean WRC between treatments indicated that SH had higher WRC ($P < 0.05$) than the other treatments. The mean WRC across cycles indicated that all three cycles differed from each other ($P < 0.05$), with Cycle One having a much higher WRC ($P < 0.0001$). Across cycles within litter types the litter in Cycle One had a significantly higher WRC ($P < 0.0001$) than the other cycles. Across treatments within cycles, litter differed from each other ($P < 0.05$) in Cycle One, but in the other cycles no differences ($P > 0.05$) were observed.

Table 4.3.5.7. Water-releasing capacity (%) of litter after five hours at Day 31

Litter type	Cycle 1	Cycle 2	Cycle 3	Mean across litter types
Biosecure shavings	$7.07^{a,A}$	$4.34^{a,B}$	$2.63^{a,B}$	4.68
Pine shavings	$7.44^{a,A}$	$3.93^{ab,B}$	$2.38^{a,C}$	4.58
Sunflower hulls	$7.34^{a,A}$	$3.73^{b,B}$	$2.45^{a,C}$	4.51
Mean across cycles	7.28^{X}	4.00^{Y}	2.49^{Z}	

^{a,b} Within columns, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{A,B,C} Within rows, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{X,Y,Z} Across cycles, means with no common superscripts differ significantly ($P < 0.05$) from each other.

After five hours at Day 31, no difference ($P > 0.05$) was found between treatments when the mean WRC across litter types was investigated. The mean WRC of litter across cycles indicated that there were differences ($P < 0.0001$) in WRC between all three cycles. Within treatments across cycles, all treatments in Cycle One had a significantly ($P < 0.05$) higher WRC when compared with the other cycles. The PS and SH both had the lowest ($P < 0.05$) WRC in Cycle Three. Within cycles across treatments, the only difference found occurred in Cycle Two, where BS had the highest WRC ($P < 0.05$), followed by PS and lastly SH.

Table 4.3.5.8. Water-releasing capacity (%) of litter after 24 hours at Day 31

Litter type	Cycle 1	Cycle 2	Cycle 3	Mean across litter types
Biosecure shavings	$27.99^{a,A}$	$14.91^{a,B}$	$10.82^{a,C}$	17.91^{x}
Pine shavings	$28.19^{a,A}$	$16.10^{a,B}$	$8.70^{ab,C}$	17.66^{x}
Sunflower hulls	$24.64^{b,A}$	$13.85^{a,B}$	$5.94^{b,C}$	14.81^{y}
Mean across cycles	26.94^{X}	14.95^{Y}	8.49^{Z}	

^{a,b} Within columns, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{A,B,C} Within rows, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{x,y} Across litter types, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{X,Y} Across cycles, means with no common superscripts differ significantly ($P < 0.05$) from each other.

After 24 hours at Day 31, the mean WRC between treatments indicated that SH had higher WRC ($P < 0.05$) than the other treatments. The mean WRC of litter across cycles indicated that there were differences ($P < 0.0001$) in WRC between all three cycles. Across treatments within cycles SH had a higher WRC ($P < 0.05$) in Cycle One when compared to the other treatments, and no difference ($P < 0.05$) was observed across litter types in Cycle Two. In Cycle Three, the highest WRC was observed in BS ($P < 0.05$) when compared to SH and PS had an intermediate WRC ($P > 0.05$) to the other treatments. Within treatments across cycles, differences were found ($P < 0.05$) in all three litter types across cycles, and litter in Cycle One had a higher WRC overall ($P < 0.0001$).

No graph of the WRC could be generated because the mean WRC across days in the production cycle did not resemble either a linear or a quadratic model.

4.4 Litter proximate analysis

4.4.1 Dry matter

Table 4.4.1.1. Litter dry matter (%) at Day Zero

Litter type	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Mean across litter types**
Biosecure shavings	$95.97^{a,A}$	$94.88^{a,B}$	$95.53^{a,AB}$	95.46^{x}
Pine shavings	$95.91^{a,A}$	$94.63^{a,B}$	$96.17^{ab,A}$	95.57^{x}
Sunflower hulls	$96.44^{a,A}$	$95.93^{b,A}$	$96.50^{b,A}$	96.29^{y}
Mean across cycles	96.11^{X}	95.14^{Y}	96.07^{X}	

^{a,b} Within columns, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{A,B} Within rows, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{x,y} Across litter types, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{X,Y} Across cycles, means with no common superscripts differ significantly ($P < 0.05$) from each other.

At Day Zero, the mean litter dry matter (DM) across treatments indicated that SH litter contained less moisture ($P < 0.05$) than the other treatments. The mean litter DM across cycles indicated that litter in Cycle Two contained more moisture ($P < 0.05$) than litter in the other cycles. Across treatments within cycles no difference ($P > 0.05$) in DM was observed in Cycle One. In Cycle Two, SH had less moisture ($P < 0.05$) than the other treatments. In Cycle Three, BS had the most moisture ($P < 0.05$), SH had the least moisture ($P < 0.05$) and PS had a moisture content intermediate to these treatments ($P > 0.05$). Across cycles within treatments, no difference ($P > 0.05$) was reported on SH, but on PS the litter in Cycle Two contained more moisture ($P < 0.05$) when compared to the other cycles. On BS, litter in Cycle Two had the most moisture ($P < 0.05$), litter in Cycle One had the least moisture ($P < 0.05$), and litter in Cycle Three had a moisture content intermediate to the other cycles ($P > 0.05$).

Table 4.4.1.2. Litter dry matter (%) at Day Seven

Litter type	Cycle 1	Cycle 2	Cycle 3	Mean across litter types
Biosecure shavings	$95.00^{a,A}$	$95.11^{a,A}$	$94.98^{a,A}$	95.03^{x}
Pine shavings	$95.05^{a,A}$	$95.34^{ab,AB}$	$95.46^{b,B}$	95.28^{y}
Sunflower hulls	$94.32^{b,A}$	$95.49^{b,B}$	$95.88^{b,B}$	95.23^{xy}
Mean across cycles	94.79^{X}	95.31^{Y}	95.44^{Y}	

^{a,b} Within columns, means with no common superscripts differ significantly (P < 0.05) from each other.

^{A,B} Within rows, means with no common superscripts differ significantly (P < 0.05) from each other.

^{x,y} Across litter types, means with no common superscripts differ significantly (P < 0.05) from each other.

^{X,Y} Across cycles, means with no common superscripts differ significantly (P < 0.05) from each other.

At Day Seven the mean litter DM across treatments indicated that BS contained the most moisture (P < 0.05), PS the least moisture (P < 0.05), with SH having a moisture content intermediate (P > 0.05) to the other treatments. The mean litter DM across cycles indicated that litter in Cycle One contained more moisture (P < 0.05) than litter in the other cycles. Across treatments within cycles, SH in Cycle One and BS in Cycle Three each contained more moisture (P < 0.05) when compared with other treatments in the respective cycles. In Cycle Two, BS contained the most moisture (P < 0.05), SH the least moisture (P < 0.05), with PS having a moisture content intermediate (P > 0.05) to the other treatments. Across cycles within treatments, no difference (P > 0.05) was reported on moisture content in BS, but SH had more (P < 0.0001) moisture in Cycle One when compared to the other cycles. In Cycle Two, BS contained the most moisture (P < 0.05), SH the least moisture (P < 0.05) and PS had a moisture content intermediate (P > 0.05) to the other treatments.

Table 4.4.1.3. Litter dry matter (%) at Day 14

Litter type	Cycle 1	Cycle 2	Cycle 3	Mean across litter types
Biosecure shavings	$93.70^{a,A}$	$94.45^{a,AB}$	$94.77^{a,B}$	94.31^{x}
Pine shavings	$93.71^{a,A}$	$93.60^{b,A}$	$95.08^{a,B}$	94.13^{x}
Sunflower hulls	$93.18^{a,A}$	$93.83^{ab,A}$	$93.42^{b,A}$	93.47^{y}
Mean across cycles	93.53^{X}	93.96^{XY}	94.42^{Y}	

^{a,b} Within columns, means with no common superscripts differ significantly (P < 0.05) from each other.

^{A,B} Within rows, means with no common superscripts differ significantly (P < 0.05) from each other.

^{x,y} Across litter types, means with no common superscripts differ significantly (P < 0.05) from each other.

^{X,Y} Across cycles, means with no common superscripts differ significantly (P < 0.05) from each other.

At Day 14 the mean litter DM across treatments indicated that SH litter contained more moisture ($P < 0.05$) than the other treatments. The mean litter DM across cycles indicated that litter in Cycle One contained the most moisture ($P < 0.05$), litter in Cycle Three the least moisture ($P < 0.05$), and litter in Cycle Two had a moisture content intermediate ($P > 0.05$) to the other treatments. Across treatments within cycles no difference ($P > 0.05$) was reported in Cycle One, but in Cycle Two, PS contained the most moisture ($P < 0.05$), BS had the least moisture ($P < 0.05$) and SH had an intermediate moisture content. In Cycle Three, SH contained more moisture when compared with the other treatments. Across cycles within treatments, BS in Cycle One had the most moisture ($P < 0.05$), BS in Cycle Three the least moisture ($P < 0.05$) and BS in Cycle Two had intermediate moisture content. There was no difference ($P < 0.05$) across cycles on SH and PS had less moisture ($P < 0.05$) in Cycle Three.

Table 4.4.1.4. Litter dry matter (%) at Day 31

Litter type	Cycle 1	Cycle 2	Cycle 3	Mean across litter types
Biosecure shavings	94.65^{A}	78.66^{B}	91.01^{AB}	88.11
Pine shavings	82.78^{A}	83.47^{A}	85.86^{A}	84.04
Sunflower hulls	95.32^{A}	84.91^{A}	85.82^{A}	88.68
Mean across cycles	90.92^{X}	82.35^{Y}	87.56^{XY}	

^{A,B} Within rows, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{X,Y} Across cycles, means with no common superscripts differ significantly ($P < 0.05$) from each other.

At Day 31, the mean litter DM across treatments indicated no differences ($P > 0.05$) between the litter treatments. The mean litter DM across cycles indicated that litter in Cycle Two contained the most moisture ($P < 0.05$), litter in Cycle One the least moisture ($P < 0.05$), with litter in Cycle Three having a moisture content intermediate ($P > 0.05$) to the other treatments. Across treatments within cycles no difference ($P > 0.05$) was reported in any of the treatments. Across cycles within treatments, no differences ($P > 0.05$) were reported on either PS or SH across the cycles. With BS, litter Cycle Two contained the most moisture ($P < 0.05$), litter in Cycle One the least moisture ($P < 0.05$), and litter in Cycle Three had a moisture content intermediate ($P > 0.05$) to those in the other cycles.

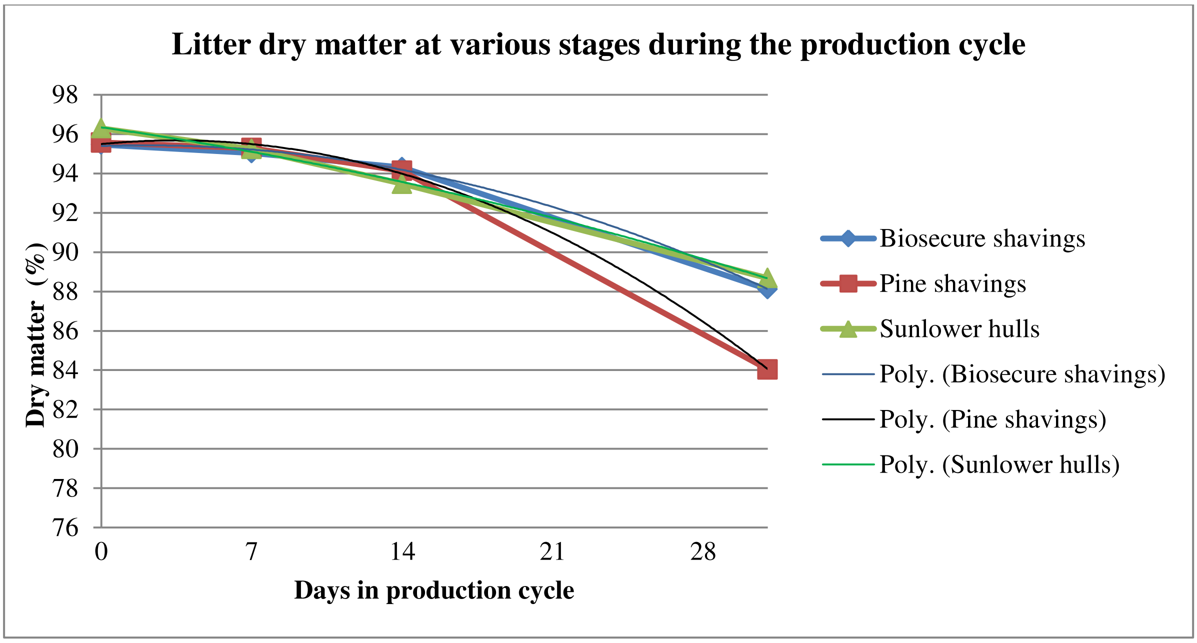

Figure 4.4.1. Litter dry matter at various stages during the production cycle

Table 4.4.1.5. Comparison of lines of best fit, R^2, and P-values of the different litter types

Litter type	**Equation**	$\mathbf{R^2}$	**P-value**
Biosecure shavings	$y = -0.009x^2 + 0.04x + 95.35$	0.9986	0.0377
Pine shavings	$y = -0.016x^2 - 0.12x + 95.38$	0.9994	0.0248
Sunflower hulls	$y = -0.002x^2 - 0.18x + 96.51$	0.9996	0.0203

The above graph depicting litter dry matter over time gives a polynomial equation for the decline in dry matter percentage over a production cycle with PS showing the largest decline in litter dry matter and BS and SH had very similar curves.

4.4.2 Crude protein

Table 4.4.2.1. Litter crude protein (%) at Day Zero (Dry matter basis)

Litter type	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Mean across litter types**
Biosecure shavings	$0.83^{a,AB}$	$0.97^{a,A}$	$0.71^{a,B}$	0.84^{x}
Pine shavings	$0.98^{a,A}$	$0.90^{a,AB}$	$0.76^{a,B}$	0.88^{x}
Sunflower hulls	$6.43^{b,A}$	$6.34^{b,A}$	$6.32^{b,A}$	6.36^{y}
Mean across cycles	2.75^{X}	2.74^{X}	2.59^{Y}	

^{a,b} Within columns, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{A,B} Within rows, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{x,y} Across litter types, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{X,Y} Across cycles, means with no common superscripts differ significantly ($P < 0.05$) from each other.

At Day Zero, the mean crude protein (CP) across treatments indicated that SH contained significantly more CP ($P < 0.0001$) than the other treatments. The mean CP across cycles indicated that litter in Cycle Three contained more CP ($P < 0.05$) than the other cycles. Across litter types within cycles, SH contained more CP ($P < 0.0001$) than the other treatments in all three cycles. Across cycles within litter types, BS in Cycle Two contained the most CP ($P < 0.05$), BS in Cycle Three the least CP ($P < 0.05$), with litter in Cycle One having a CP content intermediate ($P > 0.05$) to the other cycles. The PS had the most CP ($P < 0.05$) in Cycle One, the least CP ($P < 0.05$) in Cycle Three, with litter in Cycle Two having a CP content intermediate ($P > 0.05$) to the other cycles.

Table 4.4.2.2. Litter crude protein (%) at Day Seven (Dry matter basis)

Litter type	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Mean across litter types**
Biosecure shavings	10.62^{a}	12.46^{a}	12.01^{a}	11.69^{x}
Pine shavings	6.73^{b}	9.46^{b}	9.16^{a}	8.45^{y}
Sunflower hulls	11.68^{a}	12.07^{ab}	11.67^{a}	11.80^{x}
Mean across cycles	9.67	11.33	10.95	

^{a,b} Within columns, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{x,y} Across litter types, means with no common superscripts differ significantly ($P < 0.05$) from each other.

At Day Seven, the mean CP across treatments indicated that PS contained less CP ($P < 0.05$) than the other treatments. The mean CP across cycles indicated no difference ($P > 0.05$) between litter CP in the different cycles. Across cycles within litter types, no difference ($P > 0.05$) was found between the cycles on any of the treatments. Across treatments within cycles, no difference ($P > 0.05$) was found between the treatments in Cycle Three, but in Cycle One, PS had a higher CP content ($P < 0.05$) when compared with the other treatments. In Cycle Two, BS had the most CP ($P < 0.05$), PS had the least CP ($P < 0.05$), and SH had an intermediate CP content to these treatments ($P > 0.05$).

Table 4.4.2.3. Litter crude protein (%) at Day 14 (Dry matter basis)

Litter type	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Mean across litter types**
Biosecure shavings	21.34^{a}	19.73^{a}	20.33^{a}	20.47^{x}
Pine shavings	17.58^{b}	17.66^{a}	19.14^{a}	18.13^{y}
Sunflower hulls	18.53^{ab}	18.34^{a}	18.53^{a}	18.47^{y}
Mean across cycles	19.15	18.58	19.33	

^{a,b} Within columns, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{x,y} Across litter types, means with no common superscripts differ significantly ($P < 0.05$) from each other.

At Day 14 the mean CP across treatments indicated that BS contained more CP ($P < 0.05$) than the other treatments. The mean CP across cycles indicated no difference ($P > 0.05$) between litter CP in the different cycles. Across cycles within litter types, no difference ($P > 0.05$) was found between the cycles on any of the treatments. Across treatments within cycles, no differences ($P > 0.05$) were found between the treatments in cycles Two and Three, but in Cycle One BS had the most CP ($P < 0.05$), PS had the least CP ($P < 0.05$) and SH had an intermediate CP content to these treatments ($P > 0.05$).

Table 4.4.2.4. Litter crude protein (%) at Day 31 (Dry matter basis)

Litter type	Cycle 1	Cycle 2	Cycle 3	Mean across litter types
Biosecure shavings	29.47	32.25	27.51	29.74
Pine shavings	27.83	30.04	28.48	28.78
Sunflower hulls	25.56	31.01	27.05	27.87
Mean across cycles	27.62^{X}	31.10^{Y}	27.68^{XY}	

^{X,Y} Across cycles, means with no common superscripts differ significantly ($P < 0.05$) from each other

At Day 31 the mean CP across treatments indicated no difference ($P > 0.05$) between litter CP of different treatments. The mean litter CP across cycles indicated that litter in Cycle Two contained the most CP ($P < 0.05$), litter in Cycle One the least CP ($P < 0.05$), with litter in Cycle Three having a CP content intermediate ($P > 0.05$) to the other treatments. No differences ($P > 0.05$) were found within treatments across cycles or within cycles across treatments on CP levels.

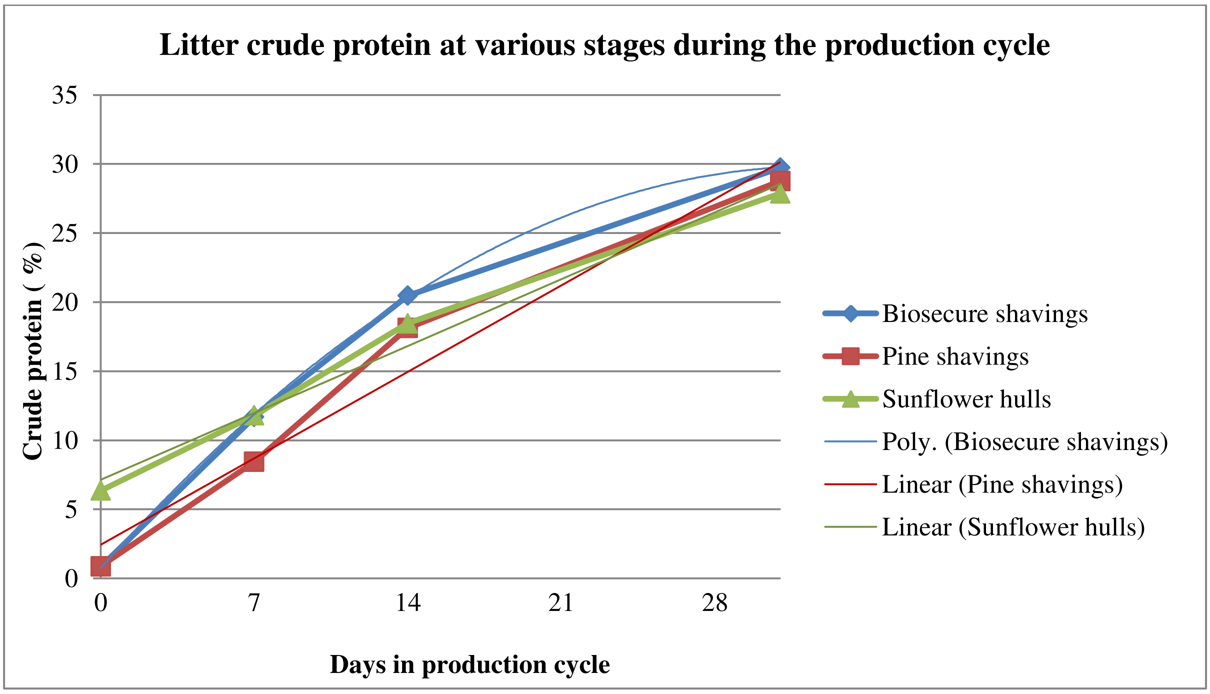

Figure 4.4.2. Litter crude protein at various stages during the production cycle

Table 4.4.2.5. Comparison of lines of best fit, R^2, and P-values of the different litter types

Litter type	Equation	R^2	P-value
Biosecure shavings	$y = -1.015x^2 + 2.01x - 1.02$	0.9997	0.0162
Pine shavings	$y = 0.91x + 1.96$	0.9597	0.0203
Sunflower hulls	$y = 0.71x + 6.76$	0.9799	0.0101

The graph indicates that the litter CP increased during the course of the production cycle. The line of best fit of BS is quadratic, but a linear line fitted PS and SH the best.

4.4.3 Acid detergent fibre

Table 4.4.3.1. Acid detergent fibre (%) of litter at the commencement of each production cycle (Dry matter basis)

Litter type	Cycle 1	Cycle 2	Cycle 3	Mean across litter types
Biosecure shavings	$84.08^{a,A}$	$83.60^{a,A}$	$84.09^{a,A}$	83.92^{x}
Pine shavings	$81.31^{b,A}$	$82.24^{a,A}$	$83.22^{a,A}$	82.26^{y}
Sunflower hulls	$64.99^{c,A}$	$63.44^{b,AB}$	$62.31^{b,B}$	63.58^{z}
Mean across cycles	76.79	76.43	76.54	

^{a,b,c} Within columns, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{A,B} Within rows, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{x,y,z} Across litter types, means with no common superscripts differ significantly ($P < 0.05$) from each other.

At the commencement of each production cycle, the mean ADF across litter types indicated differences between all litter types, with BS and PS differing from each other ($P < 0.05$) and SH had a much lower ADF content than the other litter types ($P < 0.0001$). The mean ADF across cycles indicated no difference ($P > 0.05$) between litter ADF in the different cycles. Across treatments within cycles in Cycle One all three litter types differed from each other ($P < 0.05$) with SH having much lower ADF content ($P < 0.0001$) than the other treatments across all cycles. In Cycles Two and Three, BS and PS did not differ ($P > 0.05$) in ADF content. Within treatments across cycles, no differences ($P > 0.05$) were observed in BS or PS, but SH had the most ADF in Cycle One ($P < 0.05$), the least ADF in Cycle Three ($P < 0.05$) and Cycle Two had an ADF content intermediate ($P > 0.05$) to the other cycles.

Table 4.4.3.2. Acid detergent fibre (%) of litter at the conclusion of each production cycle (Dry matter basis)

Litter type	Cycle 1	Cycle 2	Cycle 3	Mean across litter types
Biosecure shavings	31.73 ^{a}	29.40 ^{a}	33.59 ^{a}	31.57 ^{x}
Pine shavings	41.06 ^{b}	34.35 ^{a}	38.11 ^{a}	37.84 ^{y}
Sunflower hulls	37.83 ^{ab}	31.08 ^{a}	36.54 ^{a}	35.15 ^{xy}
Mean across cycles	36.87 ^{X}	31.61 ^{Y}	36.08 ^{XY}	

$^{a,\,b}$ Within columns, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{x,y} Across litter types, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{X,Y} Across cycles, means with no common superscripts differ significantly ($P < 0.05$) from each other.

At the conclusion of each production cycle, the mean acid detergent fibre (ADF) across treatments indicated that PS contained the most ADF ($P < 0.05$), BS the least ADF ($P < 0.05$) and SH had an ADF content intermediate ($P > 0.05$) to the other treatments. The mean ADF across cycles indicated that litter in Cycle One contained the most ADF ($P < 0.05$), litter in Cycle Two the least ADF ($P < 0.05$), and litter in Cycle Three had an ADF content intermediate ($P > 0.05$) to those in the other cycles. Across cycles within litter types, no differences ($P > 0.05$) were found in any of the treatments. Across litter types within cycles, no differences ($P > 0.05$) were found in cycles Two or Three. In Cycle One PS contained the most ADF ($P < 0.05$), BS the least ADF ($P < 0.05$) and SH had an ADF content intermediate ($P > 0.05$) to the other treatments.

4.4.4 Ether extract

Table 4.4.4.1. Ether extract (%) of litter at the commencement of each production cycle (Dry matter basis)

Litter type	Cycle 1	Cycle 2	Cycle 3	Mean across litter types
Biosecure shavings	0.78 ^{a,A}	0.53 ^{a,A}	0.61 ^{a,A}	0.64 ^{x}
Pine shavings	1.77 ^{b,A}	0.67 ^{a,B}	0.73 ^{a,B}	1.06 ^{x}
Sunflower hulls	9.42 ^{c,A}	8.85 ^{b,A}	7.19 ^{b,B}	8.49 ^{y}
Mean across cycles	3.99 ^{X}	3.35 ^{Y}	2.85 ^{Z}	

^{a,b,c} Within columns, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{A,B} Within rows, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{x,y} Across litter types, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{X,Y,Z} Across cycles, means with no common superscripts differ significantly ($P < 0.05$) from each other.

At the commencement of each production cycle, the mean ether extract (EE) across treatments indicated that SH had a high EE ($P < 0.0001$) value when compared to the other litter types. The mean

EE across cycles indicated significant differences ($P < 0.05$) between EE values of all three cycles. Across litter types within cycles, SH had a higher EE value ($P < 0.0001$) when compared with the other litter types. In Cycle One, there were differences ($P < 0.05$) between all treatments. Across cycles within litter types, no difference ($P > 0.05$) was observed in the EE values of BS, but PS had a higher EE value ($P < 0.05$) in Cycle One when compared to the other cycles and SH had a lower EE value ($P < 0.05$) in Cycle Three.

Table 4.4.4.2. Ether extract (%) of litter at the conclusion of each production cycle (Dry matter basis)

Litter type	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Mean across litter types**
Biosecure shavings	1.27	2.48	1.35	1.70
Pine shavings	1.66	2.48	1.76	1.97
Sunflower hulls	1.37	2.53	1.95	1.95
Mean across cycles	1.44 ^{X}	2.50 ^{Y}	1.69 ^{XY}	

^{X,Y} Across cycles, means with no common superscripts differ significantly ($P < 0.05$) from each other.

At the conclusion of each production cycle, the mean EE across treatments indicated no difference ($P > 0.05$) between treatments. The mean EE across cycles indicated that litter in Cycle One had the lowest EE ($P < 0.05$), litter in Cycle Two had the highest EE ($P < 0.05$), and litter in Cycle Three had an EE value intermediate ($P > 0.05$) to the former values. No differences ($P > 0.05$) were found within litter types across cycles or within cycles across litter types.

4.4.5 Ash

Table 4.4.5.1. Ash (inorganic material %) of litter at the commencement of each production cycle (Dry matter basis)

Litter type	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Mean across litter types**
Biosecure shavings	0.25 ^{a,A}	1.59 ^{a,A}	0.58 ^{a,A}	0.83 ^{x}
Pine shavings	0.46 ^{a,A}	0.49 ^{a,A}	0.49 ^{a,A}	0.48 ^{x}
Sunflower hulls	3.04 ^{b,A}	3.18 ^{b,A}	7.23 ^{b,B}	4.48 ^{y}
Mean across cycles	1.25 ^{X}	1.75 ^{X}	2.77 ^{Y}	

^{a,b} Within columns, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{A,B} Within rows, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{x,y} Across litter types, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{X,Y} Across cycles, means with no common superscripts differ significantly ($P < 0.05$) from each other.

At the commencement of each production cycle, the mean ash content across treatments indicated that SH contained more inorganic material ($P < 0.05$) than the other treatments. The mean ash content across cycles indicated that litter in Cycle Three contained more inorganic material ($P < 0.05$) than the other cycles. Across treatments within cycles, SH had higher inorganic matter content ($P < 0.05$) than the other treatments in all three cycles, and a highly significant difference ($P < 0.0001$) in Cycle Three. Across cycles within treatments, both BS and PS had no difference in ash content ($P > 0.05$) across the cycles, but SH had higher ash content in Cycle Three.

Table 4.4.5.2. Ash (inorganic material %) of litter at the conclusion of each production cycle (Dry matter basis)

Litter type	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Mean across litter types**
Biosecure shavings	13.28^{A}	15.95^{B}	13.76^{AB}	14.33
Pine shavings	12.00^{A}	15.74^{B}	12.20^{A}	13.31
Sunflower hulls	11.65^{A}	14.36^{B}	12.80^{AB}	12.94
Mean across cycles	12.31^{X}	15.35^{Y}	12.92^{X}	

^{A,B} Within rows, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{X,Y} Across cycles, means with no common superscripts differ significantly ($P < 0.05$) from each other.

At the conclusion of each production cycle, the mean ash content across litter types indicated no difference ($P > 0.05$) between treatments. The mean ash content across cycles indicated that litter in Cycle Two contained more inorganic material ($P < 0.05$) than the other cycles. Across treatments within cycles, no difference ($P > 0.05$) was found between treatments. Across cycles within treatments, both BS and SH contained the most ash ($P < 0.05$) in Cycle Two, the least ash in Cycle One ($P < 0.05$), with litter in Cycle Three having an ash content intermediate ($P > 0.05$) to the other cycles. The PS had more ash in Cycle Two when compared to the other cycles.

4.5 Litter beetle activity

Table 4.5.1.1. Comparison of total amount of worm activity (%) on different litter types across the production cycles

Litter type	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Total across litter types**
Biosecure shavings	0.45	2.64	28.43	31.52
Pine shavings	1.49	15.91	27.92	45.32
Sunflower hulls	0.06	9.49	13.62	23.16
Total across cycles	1.99	28.04	69.97	100.00

Worm activity differed ($P < 0.0001$) between litter types. The most worms occurred on PS, whereas the least worms occurred on SH.

Table 4.5.1.2. Comparison of total amount of pupae activity (%) on different litter types across the production cycles

Litter type	Cycle 1	Cycle 2	Cycle 3	Total across litter types
Biosecure shavings	0.90	7.46	27.53	35.89
Pine shavings	2.00	8.07	21.70	31.77
Sunflower hulls	0.59	14.48	17.27	32.34
Total across cycles	3.48	30.01	66.51	100

Pupae activity differed ($P < 0.0001$) between litter types. The most pupae occurred on BS whereas the least worms occurred on PS.

Table 4.5.1.3. Comparison of total amount of beetle activity (%) on different litter types across the production cycles

Litter type	Cycle 1	Cycle 2	Cycle 3	Total across litter types
Biosecure shavings	0.74	1.96	27.01	29.72
Pine shavings	0.44	6.97	31.72	39.13
Sunflower hulls	0.68	9.75	20.72	31.14
Total across cycles	1.86	18.69	79.45	100.00

Beetle activity differed ($P < 0.0001$) between litter types. The most beetles occurred on PS whereas the least worms occurred on BS.

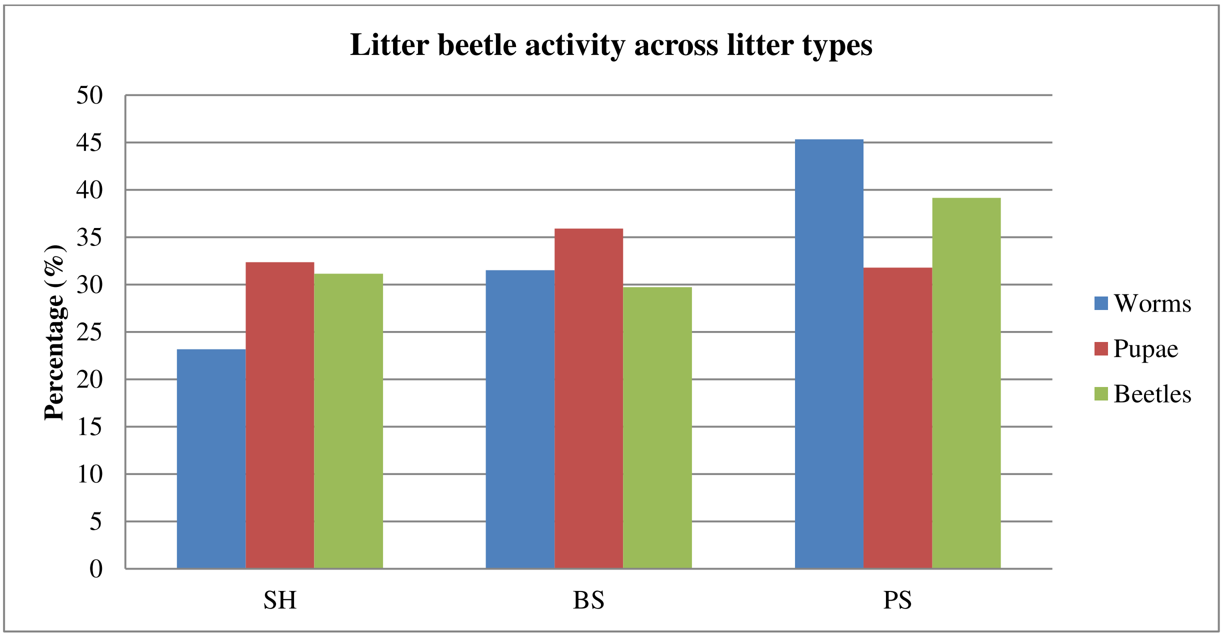

Figure 4.5.1.1. Litter beetle activity across litter types

When comparing across litter types over the three cycles, the most worms and beetles were found on PS, which was also the litter type with the highest overall beetle activity. The least worms occurred on SH, whereas the least beetles occurred on BS.

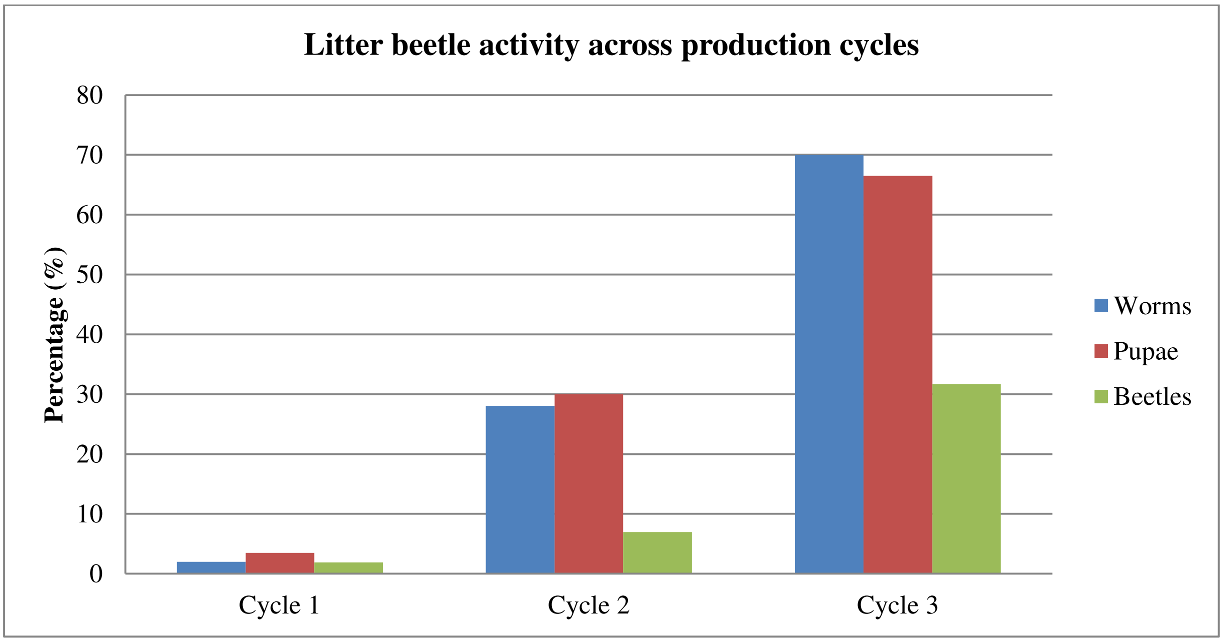

Figure 4.5.1.2. Litter beetle activity across production cycles

The above graph clearly indicates that beetle activity increased with each consecutive production cycle.

Table 4.5.2.1. Comparison of total amount of worm activity (%) in different areas of the broiler houses across litter types over three cycles

Litter type	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**Total across litter**
Biosecure shavings	2.04	3.87	1.34	2.80	5.27	4.91	0.50	2.63	3.13	5.04	31.52
Pine shavings	4.17	3.54	1.74	1.26	3.28	4.42	15.39	5.77	4.06	1.68	45.32
Sunflower hulls	2.21	1.64	0.54	0.67	2.81	3.48	3.18	1.70	3.43	3.50	23.16
Total across cycles	8.42	9.05	3.63	4.73	11.36	12.81	19.06	10.1	10.62	10.22	100.00

Differences existed (P < 0.0001) between positions in the houses during the worm stage of the beetles' lives. The most worms occurred at Position G under the feed hopper.

Table 4.5.2.2. Comparison of total amount of pupae activity (%) in different areas of the broiler houses across litter types

Litter type	A	B	C	D	E	F	G	H	I	J	Total across litter
Biosecure Shavings	2.26	1.71	1.93	0.78	9.38	10.05	0.50	3.00	3.59	2.71	35.89
Pine Shavings	1.14	4.10	0.83	0.74	9.55	5.76	0.72	2.34	5.62	0.97	31.77
Sunflower hulls	6.50	2.14	0.21	0.29	9.43	5.50	2.03	2.10	2.60	1.53	32.34
Total across cycles	9.89	7.95	2.97	1.81	28.36	21.31	3.26	7.45	11.81	5.21	100.00

Differences existed (P < 0.0001) between positions in the houses during the pupae stage of the beetles' lives. The most pupae occurred at Position E under the feeder line in the centre of the house.

Table 4.5.2.3. Comparison of total amount of beetle activity (%) in different areas of the broiler houses across litter types

Litter type	A	B	C	D	E	F	G	H	I	J	Total across litter
Biosecure shavings	3.89	2.10	2.23	0.98	5.08	2.95	2.27	4.30	2.23	3.69	29.72
Pine shavings	4.43	2.20	1.62	0.68	3.08	2.30	3.72	9.95	3.22	7.92	39.13
Sunflower hulls	2.54	1.42	2.17	1.08	5.38	2.06	1.93	2.57	6.30	5.69	31.14
Total across cycles	10.87	5.72	6.03	2.74	13.54	7.31	7.92	16.82	11.75	17.30	100.00

Differences existed ($P < 0.0001$) between positions in the houses during the beetle stage of the beetles' lives. The most beetles occurred at Position J, close to the wall on the half-house brooding side, closely followed by Position H, which is also along the same wall of the house. For descriptions of positions, refer to Figure 3.4.

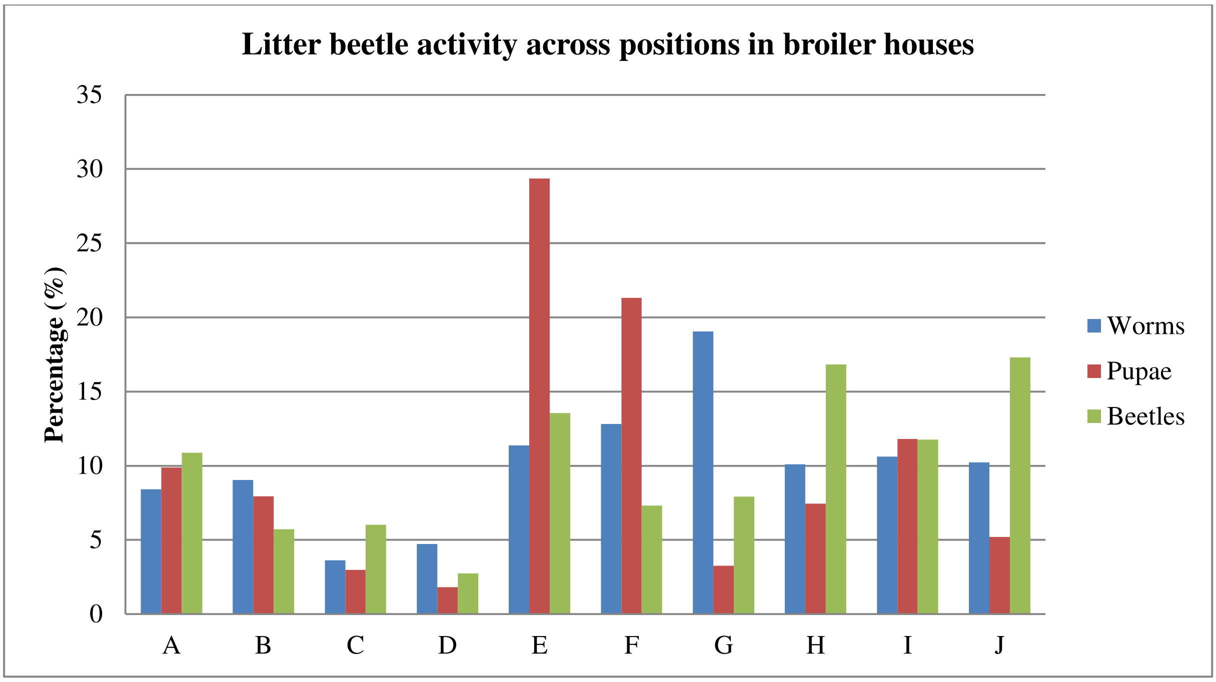

Figure 4.5.2. Litter beetle activity across positions in broiler houses

The above figure indicates that the least overall beetle activity was observed in Position D under the motor of the feed line, while the most overall beetle activity was observed at Position E under the feed line.

4.6 Footpad dermatitis scoring

Table 4.6.1. Mean footpad dermatitis scores of broilers at 21 days across the production cycles

Litter type	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Mean across litter types**
Biosecure shavings	1.825^{A}	2.413^{A}	2.125^{A}	2.121
Pine shavings	1.150^{A}	2.713^{B}	1.900^{C}	1.921
Sunflower hulls	1.600^{A}	2.138^{A}	2.250^{A}	1.996
Mean across cycles	1.525^{X}	2.421^{Y}	2.092^{Y}	

^{A,B,C} Within rows, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{X,Y} Across cycles, means with no common superscripts differ significantly ($P < 0.05$) from each other.

No differences ($P > 0.05$) were found on the mean FPD score of 21 day-old broilers across treatments. The mean FPD score of 21-day-old broilers across cycles indicated that broilers had a significantly lower FPD score ($P < 0.05$) during Cycle One, as compared to the other cycles. Within cycles across treatments, no differences ($P > 0.05$) were observed between the different treatments during any of the cycles. Within treatments across cycles, no differences ($P > 0.05$) were observed on either BS or SH, but on PS, all three cycles differed ($P < 0.05$) from each other.

Table 4.6.2. Mean footpad dermatitis scores of broilers at 31 days

Litter type	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Mean across litter types**
Biosecure shavings	$3.238^{a,A}$	$3.638^{a,A}$	$3.425^{a,A}$	3.433
Pine shavings	$2.100^{b,A}$	$3.488^{a,B}$	$3.638^{a,B}$	3.075
Sunflower hulls	$3.050^{a,A}$	$3.850^{a,B}$	$3.550^{a,AB}$	3.483
Mean across cycles	2.796^{X}	3.658^{Y}	3.538^{Y}	

^{a,b} Within columns, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{A,B} Within rows, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{X,Y} Across cycles, means with no common superscripts differ significantly ($P < 0.05$) from each other

At 31 days, no differences ($P > 0.05$) were found on the mean FPD score of 31-day-old broilers across treatments. The mean FPD score of 31-day-old broilers across cycles indicated that broilers had a significantly lower FPD score ($P < 0.05$) during Cycle One, compared to the other cycles. Within cycles across treatments, no differences ($P > 0.05$) were observed between the different treatments during cycles Two and Three, but during Cycle One, broilers on PS had lower ($P < 0.05$) FPD scores compared to the other treatments. Within treatments across cycles, no difference ($P > 0.05$) was observed in FPD scores of broilers on BS, but on PS, broilers had lower FPD scores ($P < 0.05$) during Cycle One as compared to the other cycles. Broilers provided with SH in Cycle One had the lowest FPD score ($P < 0.05$), those during Cycle Two had the highest FPD score ($P < 0.05$), with those in Cycle Three having an intermediate FPD score ($P > 0.05$) to the other cycles.

Table 4.6.3. Mean footpad dermatitis score on different litter types at 21 and 31 days

Litter type	**21 days**	**31 days**
Biosecure shavings	2.121^{X}	3.433^{Y}
Pine shavings	1.921^{X}	3.075^{Y}
Sunflower hulls	1.996^{X}	3.483^{Y}

^{X,Y} Within rows, means with no common superscripts differ significantly ($P < 0.05$) from each other.

Between litter types, no difference was recorded on a single day, but between days, all FPD scores differed significantly ($P < 0.0001$).

4.7 Production parameters

4.7.1 Total feed consumed by broilers

Table 4.7.1. Comparison of total feed consumed (kg) of 33-day-old broilers on different litter treatments over three production cycles

Litter type	Cycle 1	Cycle 2	Cycle 3	Mean across litter types
Biosecure shavings	119763.5 ^{a,A}	118121.5 ^{a,A}	112887.0 ^{a,A}	116924.3
Pine shavings	110940.0 ^{b,A}	124530.0 $^{ab, B}$	117952.0 $^{ab, AB}$	116924.0
Sunflower hulls	111236.5 ^{b,A}	128045.0 ^{b,B}	123600.0 ^{b,B}	120960.5
Mean across cycles	113980.0 ^{X}	123565.5 ^{Y}	118146.3 ^{X}	

^{a,b} Within columns, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{A,B} Within rows, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{X,Y} Across cycles, means with no common superscripts differ significantly ($P < 0.05$) from each other.

No difference ($P > 0.05$) was found in the total feed consumed when the mean over litter types for 33-day-old broilers was examined. Across cycles, the amount of feed consumed in Cycle Two was higher ($P < 0.05$) than it was in the other cycles. In Cycle One, broilers consumed more feed ($P < 0.05$) when provided with BS than when compared to other treatments, and in Cycle Two, more feed ($P < 0.05$) was consumed by birds on the SH treatment. There was no significant difference ($P > 0.05$) between litter types within Cycle Three. Within litter types across cycles, no significant differences ($P > 0.05$) were found between feed consumed in the BS treatment across the three cycles. On the PS treatment, broilers consumed significantly ($P < 0.05$) more feed in Cycle Two, and on the SH treatment, less feed ($P < 0.05$) was consumed in Cycle One.

4.7.2 Kilograms of broiler meat produced per m^2

Table 4.7.2. Comparison of kilograms of broiler meat per m^2 at Day 33 of production between three production cycles on different litter types

Litter type	Cycle 1	Cycle 2	Cycle 3	Mean across litter types
Biosecure shavings	37.785 ^{a,AB}	39.405 ^{a,A}	35.655 ^{a,B}	37.615 ^{x}
Pine shavings	36.760 ^{a,A}	39.005 ^{a,A}	38.210 ^{b,A}	37.992 ^{x}
Sunflower hulls	38.965 ^{a,A}	42.135 ^{b,B}	39.800 ^{b,AB}	40.300 ^{y}
Mean across cycles	37.837 ^{X}	40.182 ^{Y}	37.888 ^{X}	

^{a,b} Within columns, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{A,B} Within rows, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{x,y} Across litter types, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{X,Y} Across cycles, means with no common superscripts differ significantly ($P < 0.05$) from each other.

When the mean kg/m^2 across treatments was investigated at Day 33, it was found that there was a higher yield ($P < 0.05$) on SH when compared to the other treatments. When the mean kg/m^2 across cycles was investigated, it was found that there was a higher yield ($P < 0.05$) in Cycle Two when compared to the other cycles. Across treatments within cycles, no difference ($P > 0.05$) was found between treatments in Cycle One, however in Cycle Two, birds provided with SH yielded more kg/m^2 ($P < 0.05$) than the other treatments and in Cycle Three, birds provided with BS yielded less kg/m^2 ($P < 0.05$) than the other treatments. Across cycles within treatments, the kg/m^2 remained consistent ($P > 0.05$) throughout the cycles with birds provided with PS. On BS, the highest yield was in Cycle Two, the lowest yield in Cycle Three ($P < 0.05$) and Cycle One was intermediate ($P > 0.05$) to these yields. On SH, the highest yield was in Cycle Two, the lowest yield in Cycle One ($P < 0.05$) and Cycle Three was intermediate ($P > 0.05$) to these yields.

4.7.3 Mean slaughter weight of broilers

Table 4.7.3. Comparison of mean slaughter weight (kg) of broilers at Day 33 of production between three production cycles on different litter types

Litter type	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Mean across litter types**
Biosecure shavings	$1.793^{a,A}$	$1.766^{a,A}$	$1.701^{a,A}$	1.753^{x}
Pine shavings	$1.723^{a,A}$	$1.780^{a,A}$	$1.820^{b,A}$	1.774^{x}
Sunflower hulls	$1.801^{a,A}$	$1.866^{a,A}$	$1.876^{b,A}$	1.848^{y}
Mean across cycles	1.772	1.804	1.799	

^{a,b} Within columns, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{A,B} Within rows, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{x,y} Across litter types, means with no common superscripts differ significantly ($P < 0.05$) from each other.

The mean slaughter weight of birds at Day 33 across treatments indicated that birds reared on SH had a larger slaughter weight ($P < 0.05$) when compared to the other treatments. The mean slaughter weight of birds across cycles did not differ ($P > 0.05$) between cycles. Across treatments within cycles, no differences were seen across treatments in Cycle One or Two, but in Cycle Three, birds on BS had a lower slaughter weight ($P < 0.05$) when compared to the other treatments. Across cycles within treatments, the slaughter weight of birds did not differ ($P > 0.05$) between cycles on any of the treatments.

4.7.4 Average daily gain of broilers

Table 4.7.4. Comparison of average daily gain of 33-day-old broilers on different litter treatments over three production cycles

Litter type	Cycle 1	Cycle 2	Cycle 3	Mean across litter types
Biosecure shavings	54.01 ^{a,A}	52.51 ^{a,AB}	50.74 ^{a,B}	52.418 ^{x}
Pine shavings	52.38 ^{a,A}	52.94 ^{a,A}	54.69 ^{b,A}	53.335 ^{x}
Sunflower hulls	54.52 ^{a,A}	56.14 ^{b,A}	55.88 ^{b,A}	55.513 ^{y}
Mean across cycles	53.63	53.86	53.77	

^{a,b} Within columns, means with no common superscripts differ significantly (P < 0.05) from each other.

^{A,B} Within rows, means with no common superscripts differ significantly (P < 0.05) from each other.

^{x,y} Across litter types, means with no common superscripts differ significantly (P < 0.05) from each other.

33-day-old broilers on SH had a significantly higher (P < 0.05) average daily gain (ADG) across litter types. There were no significant differences (P > 0.05) in ADG mean across production cycles. During Cycle One, ADG did not differ significantly (P > 0.05) between litter types. In Cycle Two, broilers provided with SH had a significantly higher (P < 0.05) ADG than broilers on other treatments, and in Cycle Three, broilers provided with BS had significantly lower (P < 0.05) ADG than broilers on the other treatments. Across cycles within BS, broilers in Cycle Three had a significantly lower (P < 0.05) ADG than broilers in the previous cycles. There were no other differences within treatments across cycles.

4.7.5. Feed conversion ratio of broilers

Table 4.7.5. Comparison of commercial feed conversion ratio of 33-day-old broilers on different litter treatments over three production cycles

Litter type	Cycle 1	Cycle 2	Cycle 3	Mean across litter types
Biosecure shavings	1.69	1.57	1.69	1.65
Pine shavings	1.61	1.70	1.65	1.65
Sunflower hulls	1.52	1.62	1.66	1.60
Mean across cycles	1.61	1.63	1.66	

There were no significant differences (P > 0.05) reported in FCR of broilers on different litter types or across cycles.

4.7.6 Production efficiency factor of broilers

Table 4.7.6. Comparison of production efficiency factor of 33-day-old broilers on different litter treatments over three production cycles

Litter type	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Mean across litter types**
Biosecure shavings	296.355	330.582	278.455	301.797
Pine shavings	354.09	300.777	307.093	320.653
Sunflower hulls	322.525	345.700	315.881	328.035
Mean across cycles	324.323	325.686	300.476	

There were no significant differences ($P > 0.05$) reported in PEF of broilers on different litter types or across cycles.

4.7.7 Mortalities at 7 days and 33 days

Table 4.7.7.1. Comparison of seven-day mortalities (%) between three production cycles on different litter types

Litter type	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Mean across litter types**
Biosecure shavings	0.880$^{a, A}$	1.055^{a,AB}	1.420$^{a, B}$	1.118^{x}
Pine shavings	0.575$^{a, A}$	1.660$^{b, A}$	1.010^{ab,A}	1.082^{x}
Sunflower hulls	0.585$^{a, A}$	0.835$^{a, A}$	0.570$^{b, A}$	0.663^{y}
Mean across cycles	0.68^{X}	1.183^{Y}	1.000^{Y}	

^{a,b} Within columns, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{A,B} Within rows, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{x,y} Across litter types, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{X,Y} Across cycles, means with no common superscripts differ significantly ($P < 0.05$) from each other.

There was a lower ($P < 0.05$) seven-day mortality mean in SH as compared to the other treatments. There were less ($P < 0.05$) seven-day mortalities during Cycle One, when the mean across cycles was investigated. Within Cycle One, seven-day mortality of the broilers did not differ ($P > 0.05$) between litter types. Within Cycle Two, broilers provided with PS had significantly higher ($P < 0.05$) seven-day mortality than the other treatments. Within Cycle Three, broilers provided with BS had significantly higher ($P < 0.05$) seven-day mortality than those provided with SH. Across cycles within treatments, Cycle Three had a significantly higher ($P < 0.05$) seven-day mortality, compared to Cycle One when BS was investigated. There were no other differences ($P > 0.05$) across cycles within treatments.

Table 4.7.7.2. Comparison of 33-day mortalities (%) between three production cycles on different litter types

Litter type	Cycle 1	Cycle 2	Cycle 3	Mean across litter types
Biosecure shavings	4.82 ^{a,A}	3.985 ^{a,A}	7.235 ^{a,B}	5.347
Pine shavings	3.655 ^{ab,A}	3.275 ^{a,A}	7.28 ^{a,B}	4.737
Sunflower hulls	2.395 ^{b,A}	3.16 ^{a,A}	6.34 ^{a,B}	3.965
Mean across cycles	3.623^{X}	3.473 ^{X}	6.952 ^{Y}	

^{a,b} Within columns, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{A,B} Within rows, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{X,Y} Across cycles, means with no common superscripts differ significantly ($P < 0.05$) from each other.

The mean mortalities across treatments showed no difference ($P > 0.05$) between litter types. The mean mortality percentage across cycles also indicated higher mortalities ($P < 0.05$) in Cycle Three. Across treatments within cycles, SH had lower ($P < 0.05$) 33-day mortalities than BS in Cycle One and no differences ($P > 0.05$) were found between treatments during the other cycles. Across cycles within treatments, mortalities were significantly higher ($P < 0.05$) across all litter types in Cycle Three.

Table 4.7.8. Summary of production parameters across the different litter types

Production parameter	Litter type			
	Biosecure shavings	**Pine shavings**	**Sunflower hulls**	**ANOVA**
Total feed consumed (kg)	116924.3	116924.0	120960.5	NS
Kilogram/m^{2}	37.615 ^{x}	37.992 ^{x}	40.3 ^{y}	*
Mean slaughter weight (Kg)	1.753 ^{x}	1.774 ^{x}	1.848 ^{y}	*
Mean daily gain (g)	52.418 ^{x}	53.335 ^{x}	55.513 ^{y}	*
Feed conversion ratio	1.65	1.65	1.60	NS
Production efficiency factor	301.797	320.653	328.035	NS
Mortalities at 7 days (%)	1.118 ^{x}	1.082 ^{x}	0.663 ^{y}	*
Mortalities at 33 days (%)	5.347	4.737	3.965	NS

* $P < 0.05$

[x,y] Across litter types, means with no common superscripts differ significantly ($P < 0.05$) from each other.

From the eight production parameters, four had differences between litter types, namely kilograms/m^2, mean slaughter weight (kg), mean daily gain (g), and mortalities at seven days. Of all the parameters with differences, SH proved to be superior to the other litter types.

4.8 Broiler gut development parameters

4.8.1. Broiler gut development of 21-day-old broilers

Table 4.8.1.1. Mean live weight (g) of broilers at 21 days

Litter type	Cycle 1	Cycle 2	Cycle 3	Mean across litter types
Biosecure shavings	$985.13^{a,A}$	$950.17^{ab,B}$	$859.47^{a,C}$	931.59^{x}
Pine shavings	$958.25^{b,A}$	$928.20^{a,B}$	$843.83^{a,C}$	910.09^{y}
Sunflower hulls	$1030.05^{c,A}$	$960.88^{b,B}$	$861.50^{a,C}$	950.81^{z}
Mean across cycles	991.14^{X}	946.42^{Y}	854.93^{Z}	

[a,b,c] Within columns, means with no common superscripts differ significantly ($P < 0.05$) from each other.

[A,B,C] Within rows, means with no common superscripts differ significantly ($P < 0.05$) from each other.

[x,y,z] Across litter types, means with no common superscripts differ significantly ($P < 0.05$) from each other.

[X,Y,Z] Across cycles, means with no common superscripts differ significantly ($P < 0.05$) from each other.

Between litter types, differences ($P < 0.05$) were found on all treatments and the lowest mean weight of broilers was found on PS. A highly significant difference ($P < 0.0001$) was found between PS and SH. Mean body weight of 21-day-old broilers had highly significant ($P < 0.0001$) differences between cycles with the mean weight decreasing from Cycle One onwards. Within cycles across litter types, broilers provided with SH had the highest ($P < 0.0001$) mean weight during Cycle One when compared to PS and BS, which also differed from each other ($P < 0.05$). During Cycle Two, birds provided with PS had the lowest BW ($P < 0.05$), those provided with SH the highest BW ($P < 0.05$) and those on BS had a BW intermediate ($P > 0.05$) to the other weights. No differences were observed across litter types in Cycle Three. Across cycles within litter types, BW in Cycle Three was lower ($P < 0.0001$) than the other cycles. The BW also differed significantly ($P < 0.05$) across cycles on the other treatments.

Table 4.8.1.2. Full proventriculus and gizzard weight (g/kg live weight) of broilers at 21 days

Litter type	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Mean across litter types**
Biosecure shavings	$37.61^{a,A}$	$32.69^{a,B}$	$36.29^{a,A}$	35.53^{x}
Pine shavings	$34.98^{a,A}$	$35.47^{b,AB}$	$37.81^{a,B}$	36.10^{xy}
Sunflower hulls	$37.24^{a,A}$	$36.83^{b,A}$	$39.28^{a,A}$	37.78^{y}
Mean across cycles	36.61^{X}	35.00^{Y}	37.79^{X}	

^{a,b} Within columns, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{A,B} Within rows, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{x,y} Across litter types, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{X,Y} Across cycles, means with no common superscripts differ significantly ($P < 0.05$) from each other.

The mean weight across treatments indicated that broilers on BS had the lowest full proventriculus and gizzard weight ($P < 0.05$), those provided with SH the highest ($P < 0.05$) and those on PS had a full proventriculus and gizzard weight intermediate ($P > 0.05$) to the other weights. The mean full proventriculus and gizzard weight of broilers at 21 days across cycles indicated that broilers had lower gizzard weights ($P < 0.05$) relative to their BW during Cycle Two. Across treatments within cycles, no differences ($P > 0.05$) were observed across treatments during Cycles One and Three. In Cycle Two, broilers provided with BS had lower gizzard weights as compared to the other treatments. Across cycles within treatments, broilers provided with BS had lower gizzard weights ($P < 0.05$) during Cycle Two compared to the other cycles, and no differences ($P > 0.05$) were observed in gizzard weights of broilers provided with SH. Broilers provided with PS had the lowest full proventriculus and gizzard weight during Cycle One ($P < 0.05$), the highest weight during Cycle Three ($P < 0.05$) and those in Cycle Two had a full proventriculus and gizzard weight intermediate ($P > 0.05$) to the other weights.

Table 4.8.1.3. Empty proventriculus and gizzard weight (g/kg live weight) of broilers at 21 days

Litter type	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Mean across litter types**
Biosecure shavings	$21.42^{a,A}$	$20.48^{a,A}$	$23.29^{a,B}$	21.73^{x}
Pine shavings	$21.34^{a,A}$	$21.72^{ab,A}$	$24.65^{b,B}$	22.57^{y}
Sunflower hulls	$21.08^{a,A}$	$22.93^{b,B}$	$25.59^{b,C}$	23.20^{y}
Mean across cycles	21.28^{X}	21.71^{X}	24.51^{Y}	

^{a,b} Within columns, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{A,B,C} Within rows, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{x,y} Across litter types, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{X,Y} Across cycles, means with no common superscripts differ significantly ($P < 0.05$) from each other.

The mean empty proventriculus and gizzard weight of broilers at 21 days across treatments indicated that broilers had lower gizzard weights ($P < 0.05$) relative to their BW when provided with BS as compared to the other treatments. The mean empty proventriculus and gizzard weight of broilers at 21 days across cycles indicated that broilers had higher gizzard weights ($P < 0.0001$) relative to their BW during Cycle Three. Across treatments within cycles, no differences were found on the different treatments during Cycle One. During Cycle Two, the empty proventriculus and gizzard weight of broilers was high when on SH ($P < 0.05$), those provided with BS had the lowest weight ($P < 0.05$) and those on PS had an empty proventriculus and gizzard weight intermediate ($P > 0.05$) to the other weights. In Cycle Three, broilers provided with BS had lower gizzard weights ($P < 0.05$) when compared to the other treatments. Across cycles within treatments, broilers on BS had a higher relative gizzard weight ($P < 0.05$) when compared to those in Cycle One, and a highly significant difference ($P < 0.0001$) to those in Cycle Two. Broilers on PS had higher relative gizzard weights ($P < 0.0001$) during Cycle Three when compared to the other cycles. Birds provided with SH had differences ($P < 0.05$) between all three cycles, with Cycle One and three differing significantly ($P < 0.0001$).

Table 4.8.1.4. Empty intestinal weight (g/kg live weight) of broilers at 21 days

Litter type	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Mean across litter types**
Biosecure shavings	42.25 ^{a,A}	27.32 ^{a,B}	32.26 ^{a,AB}	34.94
Pine shavings	27.92 ^{b,A}	29.55 ^{a,A}	31.84 ^{a,A}	29.77
Sunflower hulls	33.68 ^{ab,A}	29.12 ^{a,A}	34.18 ^{a,A}	32.33
Mean across cycles	35.62	28.66	32.76	

^{a,b} Within columns, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{A,B} Within rows, means with no common superscripts differ significantly ($P < 0.05$) from each other.

The empty intestinal weight of broilers relative to their BW at 21 days did not differ ($P > 0.05$) among the mean across cycles or across treatments. Within cycles across treatments in Cycle One, broilers on BS had the heaviest intestines ($P < 0.05$), broilers on PS had the lightest intestines ($P < 0.05$), and broilers provided with SH had intermediate intestinal weights ($P > 0.05$). No differences ($P > 0.05$) were observed in any treatments during Cycles Two and Three. Within treatments across cycles, broilers on BS had the heaviest intestines in Cycle One ($P < 0.05$), broilers in Cycle Two had the lightest intestines ($P < 0.05$), and broilers in Cycle Three had intermediate intestinal weights ($P > 0.05$). The other treatments did not have differences between cycles ($P > 0.05$).

Table 4.8.1.5. Gizzard length (mm/kg live weight) of broilers at 21 days

Litter type	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Mean across litter types**
Biosecure shavings	$51.89^{a,A}$	$48.47^{a,B}$	$54.62^{a,C}$	51.66
Pine shavings	$49.71^{ab,A}$	$49.49^{ab,A}$	$54.47^{a,B}$	51.23
Sunflower hulls	$48.19^{b,A}$	$51.76^{b,B}$	$53.92^{a,B}$	51.29
Mean across cycles	49.93^{X}	49.90^{X}	54.34^{Y}	

^{a,b}Within columns, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{A,B,C}Within rows, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{X,Y}Across cycles, means with no common superscripts differ significantly ($P < 0.05$) from each other.

The mean gizzard length of broilers at 21 days across treatments indicated no differences ($P > 0.05$) among treatments. The mean gizzard length of broilers at 21 days across cycles indicated that broilers had longer gizzards ($P < 0.0001$) relative to their BW during Cycle Three. Across treatments within cycles, during cycles One and Two, a similar pattern was observed, and no differences ($P < 0.05$) were observed during Cycle Three. Broilers provided with SH in Cycle One had longer gizzards and in Cycle Two shorter gizzards ($P < 0.05$); those provided with BS had the shortest gizzards in Cycle One and the longest in Cycle Two ($P < 0.05$); and those on PS had gizzard lengths intermediate ($P > 0.05$) to the other treatments in both cycles. Across cycles within treatments, gizzard length in broilers on BS differed across all three cycles ($P < 0.05$), with the difference between Cycle Two and Cycle Three being highly significant ($P < 0.0001$). On PS, broilers had longer gizzards ($P < 0.05$) during Cycle Three, when compared with the other cycles. On SH, broilers had shorter gizzards ($P < 0.05$) during Cycle One when compared with the other cycles.

Table 4.8.1.6. Gizzard width (mm/kg live weight) of broilers at 21 days

Litter type	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Mean across litter types**
Biosecure shavings	$36.03^{a,A}$	$32.52^{a,B}$	$39.81^{a,C}$	36.12^{x}
Pine shavings	$36.12^{a,A}$	$36.62^{b,B}$	$42.93^{a,B}$	38.56^{y}
Sunflower hulls	$33.96^{a,A}$	$37.03^{b,A}$	$42.06^{a,B}$	37.68^{xy}
Mean across cycles	35.37^{X}	35.39^{X}	41.60^{Y}	

^{a,b}Within columns, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{A,B,C}Within rows, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{x,y}Across litter types, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{X,Y}Across cycles, means with no common superscripts differ significantly ($P < 0.05$) from each other

The mean gizzard width across treatments at 21 days indicated that broilers on PS had the widest gizzards ($P < 0.05$), broilers on BS the narrowest gizzards ($P < 0.05$), and broilers had intermediate ($P > 0.05$) widths to the other treatments on SH. The mean gizzard width of broilers at 21 days across cycles indicated that broilers had wider gizzards ($P < 0.0001$) relative to their BW during Cycle Three. Across treatments within cycles, no differences ($P > 0.05$) were observed across treatments during cycles One and Three, however, in Cycle Two, broilers provided with BS had narrower gizzards ($P < 0.05$) when compared to the other treatments. Across cycles within treatments, differences were seen across all three cycles when using BS for broilers ($P < 0.05$), and a highly significant difference ($P < 0.0001$) was observed between cycles Two and Three. Broilers on PS had narrower gizzards ($P < 0.05$) during Cycle One and broilers on SH had wider gizzards ($P < 0.05$) during Cycle Three when compared to the other cycles in the respective litter types.

Table 4.8.1.7. Proventriculus sphincter width (mm/kg live weight) of broilers at 21 days

Litter type	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Mean across litter types**
Biosecure shavings	$16.11^{a,A}$	$11.65^{a,B}$	$12.20^{a,B}$	13.32^{x}
Pine shavings	$15.16^{a,A}$	$13.49^{a,A}$	$10.18^{b,B}$	12.95^{xy}
Sunflower hulls	$12.61^{b,A}$	$11.57^{a,A}$	$11.59^{ab,A}$	11.92^{y}
Mean across cycles	14.63^{X}	12.24^{Y}	11.33^{Y}	

^{a,b} Within columns, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{A,B} Within rows, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{x,y} Across litter types, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{X,Y,Z} Across cycles, means with no common superscripts differ significantly ($P < 0.05$) from each other.

The mean proventriculus sphincter width of broilers at 21 days across cycles indicated that broilers had wider proventriculus sphincters ($P < 0.0001$) relative to their BW during Cycle One. The mean proventriculus sphincter width across treatments indicated that broilers on BS had the widest proventriculus sphincters ($P < 0.05$), the narrowest sphincters ($P < 0.05$) on SH, with broilers having intermediate ($P > 0.05$) widths to the other treatments on PS. Across treatments within cycles, sphincters of birds were wider ($P < 0.05$) on SH as compared to the other treatments during Cycle One. No differences ($P > 0.05$) were reported among treatments during Cycle Two. In Cycle Three, broilers on BS had the widest proventriculus sphincters ($P < 0.05$) the narrowest sphincters ($P < 0.05$) on PS and broilers on SH had intermediate ($P > 0.05$) widths to the other treatments. Across cycles within treatments, broilers had wider proventriculus sphincters ($P < 0.05$) when reared on BS in Cycle One, and narrower sphincters ($P < 0.05$) on PS during Cycle Three when compared to the other cycles in the respective treatments. No differences ($P > 0.05$) were reported among cycles with broilers reared on SH.

Table 4.8.1.8. Duodenum and jejenum length (cm/kg live weight) of broilers at 21 days

Litter type	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Mean across litter types**
Biosecure shavings	89.18 ^{a,A}	93.71 ^{a,A}	108.64 ^{a,B}	97.18 ^{x}
Pine shavings	94.83 ^{a,A}	108.06 ^{b,B}	110.86 ^{a,B}	104.59 ^{y}
Sunflower hulls	91.19 ^{a,A}	103.73 ^{b,B}	116.24 ^{a,C}	103.72 ^{y}
Mean across cycles	91.74 ^{X}	101.84 ^{Y}	111.91 ^{Z}	

^{a,b} Within columns, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{A,B,C} Within rows, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{x,y} Across litter types, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{X,Y,Z} Across cycles, means with no common superscripts differ significantly ($P < 0.05$) from each other

The mean duodenal length across litter types at 21 days indicated that duodenum length of broilers was shorter ($P < 0.05$) on BS, compared to the other treatments. The mean duodenum and jejenum length of broilers at 21 days across cycles indicated that broilers had differences ($P < 0.0001$) between intestinal lengths relative to their BW across all three cycles, with the longest lengths observed during Cycle Three. Across treatments within cycles, no differences ($P > 0.05$) were reported among treatments during cycles One or Three, but during Cycle Two, broilers provided with BS had shorter relative intestinal lengths than PS ($P < 0.0001$) and SH ($P < 0.05$). Across cycles within treatments, broilers had longer intestines ($P < 0.0001$) when provided with BS during Cycle Three, and those provided with PS had shorter intestines ($P < 0.0002$) during Cycle One, as compared to the other cycles in the respective treatments. Broilers provided with SH had differences ($P < 0.05$) across all three cycles, with intestinal lengths of broilers in Cycle One being significantly lower ($P < 0.0001$).

Table 4.8.1.9. Ileum length (cm/kg live weight) of broilers at 21 days

Litter type	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Mean across litter types**
Biosecure shavings	62.95 ^{a,A}	74.56 ^{a,B}	84.37 ^{ab,C}	73.96
Pine shavings	64.33 ^{a,A}	79.89 ^{b,B}	80.56 ^{b,B}	74.93
Sunflower hulls	65.47 ^{a,A}	78.19 ^{ab,B}	86.56 ^{a,C}	76.74
Mean across cycles	64.25 ^{X}	77.55 ^{Y}	83.83 ^{Z}	

^{a,b} Within columns, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{A,B,C} Within rows, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{X,Y,Z} Across cycles, means with no common superscripts differ significantly ($P < 0.05$) from each other.

The mean ileum length at 21 days across litter types indicated no differences (P > 0.05) between treatments. The mean ileum length of broilers at 21 days across cycles indicated that broilers had differences (P < 0.0001) between intestinal lengths relative to their BW across all three cycles, with the longest lengths observed during Cycle Three. Across treatments within cycles, no differences were found among treatments during Cycle One. During Cycle Two, broilers had the longest ileums when on PS (P < 0.05), the shortest ileums on BS (P < 0.05) and ileums of intermediate length (P > 0.05) when on SH. During Cycle Three, broilers had the longest ileums when on SH (P < 0.05), the shortest ileums on PS (P < 0.05) and ileums of intermediate length (P > 0.05) when on BS. Across cycles within treatments, differences existed (P < 0.0001) among all three cycles on BS and SH, respectively, with relative ileal lengths being the longest during Cycle Three. On PS, broilers had shorter ileums (P < 0.05) during Cycle One as compared to the other cycles.

Table 4.8.1.10. Caecum length (cm/kg live weight) of broilers at 21 days

Litter type	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Mean across litter types**
Biosecure shavings	$12.92^{a,A}$	$15.68^{a,B}$	$17.48^{a,C}$	15.36^{x}
Pine shavings	$13.81^{b,A}$	$16.55^{a,B}$	$17.29^{a,B}$	15.89^{y}
Sunflower hulls	$13.54^{ab,A}$	$15.76^{a,B}$	$16.66^{a,B}$	15.32^{x}
Mean across cycles	13.42^{X}	16.00^{Y}	17.15^{Z}	

^{a,b} Within columns, means with no common superscripts differ significantly (P < 0.05) from each other.

^{A,B,C} Within rows, means with no common superscripts differ significantly (P < 0.05) from each other.

^{x,y} Across litter types, means with no common superscripts differ significantly (P < 0.05) from each other.

^{X,Y,Z} Across cycles, means with no common superscripts differ significantly (P < 0.05) from each other

The mean caecum length of broilers at 21 days across treatments indicated that broilers on PS had on mean longer caeca (P < 0.05) than broilers on the other treatments. The mean caecum length of broilers at 21 days across cycles indicated that broilers had differences (P < 0.0001) relative to their BW across all three cycles, with the longest relative lengths observed during Cycle Three. Across treatments within cycles, during Cycle One, broilers had the longest caeca when on PS (P < 0.05), the shortest caeca on BS (P < 0.05) and caeca of intermediate length (P > 0.05) when on SH. No differences (P > 0.05) were reported among treatments during cycles Two and Three. Across cycles within treatments, the gizzard widths differed (P < 0.0001) across all three cycles on BS with the longest proportional caeca found in Cycle Three. On PS and SH, caecum lengths during Cycle One were shorter (P < 0.0001) that during the other cycles.

Table 4.8.1.11. Caecum length (cm/kg live weight) of broilers at 21 days

Litter type	Cycle 1	Cycle 2	Cycle 3	Mean across litter types
Biosecure shavings	12.95 ^{a,A}	15.77 ^{a,B}	17.30 ^{a,C}	15.34 ^{x}
Pine shavings	14.20 ^{b,A}	16.62 ^{a,B}	17.28 ^{a,B}	16.03 ^{y}
Sunflower hulls	13.51 ^{ab,A}	15.91 ^{a,B}	17.01 ^{a,C}	15.48 ^{x}
Mean across cycles	13.55 ^{X}	16.10 ^{Y}	17.20 ^{Z}	

^{a,b} Within columns, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{A,B,C} Within rows, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{x,y} Across litter types, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{X,Y,Z} Across cycles, means with no common superscripts differ significantly ($P < 0.05$) from each other.

The mean caecum length at 21 days across treatments indicated that broilers on PS had proportionately longer caeca ($P < 0.05$) as compared to the other treatments. Differences ($P < 0.0001$) were observed in the mean caecum length of broilers at 21 days across all three cycles, with the longest relative lengths observed during Cycle Three. Across treatments within cycles, during Cycle One, broilers had the longest caeca when on PS ($P < 0.05$), the shortest caeca on BS ($P < 0.05$), and caeca of intermediate length ($P > 0.05$) when on SH. No differences ($P > 0.05$) were reported among treatments during Cycles Two and Three. Across cycles within treatments, the caecum lengths differed ($P < 0.05$) across all three cycles on BS and SH, with the longest proportional caeca found in Cycle Three. Differences between Cycle One and the other cycles were highly significant ($P < 0.0001$) on both litter types. On PS, broilers had proportionately shorter caeca during Cycle One ($P < 0.0001$) when compared to the other cycles.

Table 4.8.1.12. Gizzard content dry matter (%) of broilers at 21 days

Litter type	Cycle 1	Cycle 2	Cycle 3	Mean across litter types
Biosecure shavings	92.02 ^{a,A}	92.00 ^{a,A}	92.43 ^{a,B}	92.15 ^{x}
Pine shavings	92.02 ^{a,A}	92.15 ^{a,A}	92.64 ^{ab,B}	92.27 ^{xy}
Sunflower hulls	92.26 ^{a,A}	92.04 ^{a,A}	92.79 ^{b,B*}	92.36 ^{y}
Mean across cycles	92.10 ^{X}	92.06 ^{X}	92.62 ^{Y}	

^{a,b} Within columns, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{A,B} Within rows, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{x,y} Across litter types, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{X,Y} Across cycles, means with no common superscripts differ significantly ($P < 0.05$) from each other.

The mean dry matter of the gizzard content at 21 days across treatments indicated that gizzard content had the most moisture ($P < 0.05$) on BS, the least on SH ($P < 0.05$) and intermediate moisture content ($P > 0.05$) on PS. The mean gizzard content dry matter at 21 days across cycles indicated that Cycle Three had less moisture ($P < 0.0001$), compared to the other cycles. Across treatments within cycles, no differences ($P > 0.05$) were found during cycles One and Two. In Cycle Three gizzard moisture content was the most with birds on BS ($P < 0.05$), the least with birds on SH ($P < 0.05$) and intermediate moisture content when birds were on PS ($P > 0.05$). Across cycles within treatments, all three treatments had birds with lower gizzard moisture content ($P < 0.05$) during Cycle Three. The birds provided with PS had gizzard DM content with highly significant differences ($P < 0.0001$) between cycles One and Three. Highly significant differences ($P < 0.0001$) were observed between Cycle Three and the other two cycles when birds were provided with SH.

Table 4.8.1.13. Gizzard content acid detergent fibre (%) of broilers at 21 days

Litter type	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Mean across litter types**
Biosecure shavings	$15.51^{a,A}$	$12.69^{a,A}$	$15.35^{a,A}$	14.52^{x}
Pine shavings	$15.41^{a,A}$	$13.99^{a,A}$	$21.21^{b,B}$	16.87^{y}
Sunflower hulls	$18.04^{a,AB}$	$15.86^{a,A}$	$20.80^{b,B}$	18.23^{y}
Mean across cycles	16.32^{X}	14.18^{Y}	19.12^{Z}	

^{a,b} Within columns, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{A,B} Within rows, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{x,y} Across litter types, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{X,Y,Z} Across cycles, means with no common superscripts differ significantly ($P < 0.05$) from each other.

The mean ADF for gizzard content across treatments indicated that ADF was lower ($P < 0.05$), with birds provided with BS when compared to the other treatments. The mean gizzard content ADF differed between all three cycles ($P < 0.05$). Across treatments within cycles, no differences ($P > 0.05$) were found during either Cycle One or Two, but in Cycle Three birds provided with BS had lower gizzard ADF content ($P < 0.05$) when compared to the other treatments. Across cycles within treatments, no difference ($P > 0.05$) was found among cycles with birds provided with BS. Birds provided with PS had a higher ADF content in Cycle Three when compared to Cycle Two ($P < 0.0001$) and Cycle One ($P < 0.05$). Birds provided with SH had high gizzard ADF values ($P < 0.05$) during Cycle Three, significantly lower ADF values ($P < 0.05$) in Cycle Two, and intermediate gizzard ADF values in Cycle One ($P < 0.05$).

4.8.2 Broiler gut development parameters from 31-day old broilers in the production cycles

Table 4.8.2.1. Mean live weight (g) of broilers at 31 days

Litter type	Cycle 1	Cycle 2	Cycle 3	Mean across litter types
Biosecure shavings	$1797.20^{ab,A}$	$1807.06^{a,A}$	$1688.30^{a,B}$	1764.19^{x}
Pine shavings	$1735.94^{a,A}$	$1795.98^{a,A}$	$1764.34^{b,A}$	1765.42^{x}
Sunflower hulls	$1853.93^{b,A}$	$1786.43^{a,B}$	$1810.54^{b,AB}$	1816.97^{y}
Mean across cycles	1795.69^{X}	1796.49^{X}	1754.39^{Y}	

^{a,b} Within columns, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{A,B} Within rows, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{x,y} Across litter types, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{X,Y} Across cycles, means with no common superscripts differ significantly ($P < 0.05$) from each other.

Broilers had a higher mean BW ($P < 0.05$) when on SH, compared to the other treatments. Mean body weight of 31-day-old broilers was lower ($P < 0.05$) during Cycle Three. Within cycles across litter types in Cycle One, broilers provided with PS had the lowest BW ($P < 0.05$), broilers provided with SH the highest BW and those on BS had intermediate BW to the other treatments ($P < 0.05$). During Cycle Two, no differences ($P > 0.05$) were observed among treatments. In Cycle Three, broilers provided with BS had lower BW ($P < 0.05$) than broilers on the other treatments. Across cycles within litter types, BW on BS in Cycle Three was lower ($P < 0.05$) than the other cycles. No difference ($P > 0.05$) was seen on PS across the cycles. Broilers on SH had the lowest BW ($P < 0.05$) in Cycle Two, the highest BW ($P < 0.05$) in Cycle One and intermediate BW to the other cycles in Cycle Three ($P > 0.05$).

Table 4.8.2.2. Full proventriculus and gizzard weight (g/kg live weight) of broilers at 31 days

Litter type	Cycle 1	Cycle 2	Cycle 3	Mean across litter types
Biosecure shavings	$27.02^{ab,AB}$	$24.69^{a,A}$	$28.99^{a,B}$	26.90^{xy}
Pine shavings	$24.88^{a,A}$	$23.77^{a,A}$	$28.03^{a,B}$	25.56^{x}
Sunflower hulls	$27.71^{b,AB}$	$25.48^{a,A}$	$28.95^{a,B}$	27.38^{y}
Mean across cycles	26.54^{X}	24.64^{Y}	28.66^{Z}	

^{a,b} Within columns, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{A,B} Within rows, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{x,y}Across litter types, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{X,Y}Across cycles, means with no common superscripts differ significantly ($P < 0.05$) from each other.

Across litter types, the mean full proventriculus and gizzard weight of 31-day-old broilers was the highest ($P < 0.05$) on SH, the lowest ($P < 0.05$) on PS and intermediate to the other treatments ($P > 0.05$) on BS. The mean full proventriculus and gizzard weight of broilers at 31 days across cycles indicated differences across all three cycles ($P < 0.05$), with broilers having the lowest gizzard weights relative to their BW in Cycle Two. The difference between Cycle Two and Three was highly significant ($P < 0.0001$). Across treatments within cycles, no differences ($P > 0.05$) were observed during Cycle Two or Three. In Cycle One, the full proventriculus and gizzard weight of broilers was high when on SH ($P < 0.05$), those provided with PS had the lowest weight ($P < 0.05$), and those on BS had a full proventriculus and gizzard weight intermediate ($P > 0.05$) to the other treatments. Across cycles within treatments, on BS and SH, gizzard weight was the lowest ($P < 0.05$) in Cycle Two, the highest in Cycle Three ($P < 0.05$) and the gizzard weight in Cycle One intermediate to the weights on the other cycles ($P > 0.05$). The broilers on PS had heavier gizzards ($P < 0.05$) relative to their live weight during Cycle Three.

Table 4.8.2.3. Empty proventriculus and gizzard weight (g/kg live weight) of broilers at 31 days

Litter type	Cycle 1	Cycle 2	Cycle 3	Mean across litter types
Biosecure shavings	16.82^{A}	16.63^{A}	19.09^{B}	17.51^{x}
Pine shavings	16.77^{A}	15.59^{A}	18.17^{B}	16.84^{y}
Sunflower hulls	17.34^{AB}	16.22^{A}	18.48^{B}	17.35^{xy}
Mean across cycles	16.98^{X}	16.15^{Y}	18.58^{Z}	

^{A,B} Within rows, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{x,y} Across litter types, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{X,Y,Z} Across cycles, means with no common superscripts differ significantly ($P < 0.05$) from each other.

The mean empty proventriculus and gizzard weight of broilers at 31 days across litter types indicated that the highest weights ($P < 0.05$) were with broilers on BS, the lowest weights with broilers on PS and intermediate weights ($P > 0.05$) on SH. Mean empty proventriculus and gizzard weight of broilers at 31 days differed ($P < 0.05$) among cycles, with the weight being the highest ($P < 0.0001$) in Cycle Three as compared to the other cycles. Across treatments within cycles, no differences ($P > 0.05$) were observed on the treatments during any of the cycles. Across cycles within litter types on BS, gizzard weight was heavier ($P < 0.05$) in Cycle Three when compared with Cycle One and also heavier ($P < 0.0001$) when compared with Cycle Two. On PS, gizzard weight was heavier ($P < 0.05$) in Cycle Three when compared with the other cycles. Broilers provided with SH had the lowest gizzard weights ($P < 0.05$) in Cycle Two, the highest in Cycle Three ($P < 0.05$), and in Cycle One the gizzard weight was intermediate to those in the other cycles ($P > 0.05$).

Table 4.8.2.4. Empty intestinal weight (g/kg live weight) of broilers at 31 days

Litter type	Cycle 1	Cycle 2	Cycle 3	Mean across litter types
Biosecure shavings	$24.70^{a,A}$	$25.11^{a,A}$	$26.76^{a,A}$	25.52^{x}
Pine shavings	$22.43^{b,A}$	$22.93^{b,A}$	$26.04^{a,B}$	23.80^{y}
Sunflower hulls	$23.43^{ab,A}$	$22.17^{b,A}$	$26.23^{a,B}$	23.91^{y}
Mean across cycles	23.48^{X}	23.41^{X}	26.34^{Y}	

^{a,b} Within columns, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{A,B} Within rows, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{x,y} Across litter types, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{X,Y} Across cycles, means with no common superscripts differ significantly ($P < 0.05$) from each other.

The mean empty intestinal weight of broilers at 31 days across treatments indicated that BS had proportionately heavier intestines ($P < 0.05$) when compared to the other treatments. The mean empty intestinal weight of broilers relative to their live weight across cycles indicated that broilers in Cycle Three had proportionately heavier intestines ($P < 0.0001$) when compared to the other cycles. Across treatments within cycles, broilers housed on BS had the highest intestinal weight ($P < 0.05$), those on PS the lowest intestinal weight ($P < 0.05$), and those housed on SH had intestinal weights intermediate to the other treatments. During Cycle Two, broilers on BS had relatively heavier intestines ($P < 0.05$) as compared to the other treatments, and no differences were observed ($P > 0.05$) during Cycle Three. Across cycles within treatments, no differences ($P > 0.05$) were found across cycles on BS, but on PS and SH, the intestinal weight was proportionately higher during Cycle Three when compared to the other cycles.

Table 4.8.2.5. Gizzard length (mm/kg live weight) of broilers at 31 days

Litter type	Cycle 1	Cycle 2	Cycle 3	Mean across litter types
Biosecure shavings	$31.08^{a,AB}$	$30.58^{a,A}$	$32.73^{a,B}$	31.46
Pine shavings	$30.66^{a,A}$	$31.03^{ab,A}$	$32.38^{a,A}$	31.35
Sunflower hulls	$30.31^{a,A}$	$32.80^{b,B}$	$32.14^{a,AB}$	31.75
Mean across cycles	30.68^{X}	31.47^{XY}	32.41^{Y}	

^{a,b} Within columns, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{A,B} Within rows, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{X,Y} Across cycles, means with no common superscripts differ significantly ($P < 0.05$) from each other

The mean gizzard length of broilers at 31 days relative to their live weight across litter types indicated no difference ($P > 0.05$) between litter types. The mean gizzard length of broilers relative to their live weight across cycles indicated that broilers in Cycle One had shorter gizzards ($P < 0.05$), those in Cycle Three had longer gizzards ($P < 0.05$), and those in Cycle Two had intermediate length ($P > 0.05$), as compared to the other cycles. Across treatments within cycles, no differences ($P > 0.05$) were observed across treatments during either Cycle One or Cycle Three. During Cycle Two, broilers on BS had shorter gizzards ($P < 0.05$), those on SH had longer gizzards ($P < 0.05$), and those on PS had intermediate length gizzards ($P > 0.05$), when compared to the other treatments. Across cycles within treatments, broilers provided with BS in Cycle Two had shorter gizzards ($P < 0.05$), those in Cycle Three had longer gizzards ($P < 0.05$), and those in Cycle One had intermediate length gizzards ($P > 0.05$), as compared to birds on BS in the other cycles. Broilers provided with PS showed no differences across cycles. Broilers provided with SH in Cycle One had shorter gizzards ($P < 0.05$), those in Cycle Two had longer gizzards ($P < 0.05$), and those in Cycle Three had intermediate length gizzards ($P > 0.05$), as compared to birds on SH in the other cycles.

Table 4.8.2.6. Gizzard width (mm/kg live weight) of broilers at 31 days

Litter type	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Mean across litter types**
Biosecure shavings	$22.64^{a,A}$	$19.39^{a,B}$	$22.51^{a,A}$	21.41^{xy}
Pine shavings	$22.39^{a,A}$	$20.50^{a,B}$	$20.75^{a,B}$	21.21^{x}
Sunflower hulls	$22.49^{a,A}$	$22.38^{b,A}$	$21.81^{a,A}$	22.22^{y}
Mean across cycles	22.51^{X}	20.76^{Y}	21.59^{XY}	

^{a,b} Within columns, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{A,B} Within rows, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{x,y} Across litter types, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{X,Y} Across cycles, means with no common superscripts differ significantly ($P < 0.05$) from each other.

The mean gizzard width of broilers relative to their live weight across treatments indicated that broilers had narrower when provided with PS ($P < 0.05$), wider gizzards on SH ($P < 0.05$), and those on BS had gizzards of intermediate width ($P > 0.05$), when compared to the other treatments. The mean gizzard width of broilers relative to their live weight across cycles indicated that broilers in Cycle Two had narrower gizzards ($P < 0.05$), those in Cycle One had wider gizzards ($P < 0.05$), and those in Cycle Three had gizzards of intermediate width ($P > 0.05$), when compared to the other cycles. Across treatments within cycles, no differences ($P > 0.05$) were observed across treatments during either Cycle One or Cycle Three. During Cycle Two, broilers had wider gizzards ($P < 0.05$) when on SH when compared to the other treatments. Across cycles within treatments, broilers in Cycle Two had narrower gizzards when provided with BS, as compared to Cycle Three ($P < 0.05$) and Cycle One ($P < 0.0001$), and wider gizzards ($P < 0.05$) when provided with PS in Cycle One, as compared to the other cycles. No differences across cycles ($P > 0.05$) were observed when birds were provided with SH.

Table 4.8.2.7. Proventriculus sphincter width (mm/kg live weight) of broilers at 31 days

Litter type	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Mean across litter types**
Biosecure shavings	7.50 ^{a,A}	9.21 ^{a,B}	8.42 ^{a,AB}	8.38 ^{x}
Pine shavings	7.40 ^{a,A}	8.31 ^{a,A}	7.34 ^{b,A}	7.68 ^{y}
Sunflower hulls	7.55 ^{a,AB}	8.22 ^{a,A}	6.65 ^{b,B}	7.48 ^{y}
Mean across cycles	7.48 ^{X}	8.58 ^{Y}	7.47 ^{X}	

^{a,b} Within columns, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{A,B} Within rows, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{x,y} Across litter types, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{X,Y} Across cycles, means with no common superscripts differ significantly ($P < 0.05$) from each other.

The mean proventriculus sphincter width at 31 days across treatments indicated that broilers on BS had wider proventriculus sphincters ($P < 0.05$), when compared to the other treatments. The mean proventriculus sphincter width of broilers at 31 days across cycles indicated that broilers had wider proventriculus sphincters ($P < 0.05$) relative to their BW during Cycle Two. Across treatments within cycles, no differences ($P > 0.05$) were reported among treatments during cycles One or Two. In Cycle Three, broilers on BS had wider proventriculus sphincters ($P < 0.05$) than in the other treatments. Across cycles within treatments, broilers on BS in Cycle One had narrower proventriculus sphincters ($P < 0.05$), those in Cycle Two had wider proventriculus sphincters ($P < 0.05$), and those in Cycle Three had intermediate width ($P > 0.05$), as compared to the other cycles. No differences ($P > 0.05$) were reported among cycles with broilers reared on PS. Broilers reared on SH in Cycle Three had narrower proventriculus sphincters ($P < 0.05$), those in Cycle Two had wider proventriculus sphincters ($P < 0.05$), and those in Cycle One had intermediate width ($P > 0.05$), when compared to the other cycles.

Table 4.8.2.8. Duodenum and jejunum length (cm/kg live weight) of broilers at 31 days

Litter type	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Mean across litter types**
Biosecure shavings	63.30 ^{ab,A}	62.67 ^{a,A}	64.89 ^{a,A}	63.62
Pine shavings	67.68 ^{a,A}	61.75 ^{a,B}	62.70 ^{a,B}	64.05
Sunflower hulls	62.34 ^{b,A}	62.78 ^{a,A}	62.39 ^{a,A}	62.50
Mean across cycles	64.44	62.40	63.33	

^{a,b} Within columns, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{A,B} Within rows, means with no common superscripts differ significantly ($P < 0.05$) from each other.

The mean duodenum and jejenum length of broilers relative to their body weight at 31 days did not differ ($P > 0.05$) across cycles or across litter types. Across treatments within cycles during Cycle One, broilers on SH had shorter intestines ($P < 0.05$), those on PS had longer intestines ($P < 0.05$), and those on BS had intermediate length ($P > 0.05$), when compared to the other treatments. No differences ($P > 0.05$) were reported across treatments among cycles Two and Three. Across cycles within treatments, no differences ($P > 0.05$) were reported on BS or SH. On PS, the intestinal length of broilers was longer during Cycle One as compared to the other cycles.

Table 4.8.2.9. Ileum length (cm/kg live weight) of broilers at 31 days

Litter type	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Mean across litter types**
Biosecure shavings	49.04^{AB}	47.45^{A}	50.53^{B}	49.01^{xy}
Pine shavings	51.95^{A}	47.10^{B}	49.35^{AB}	49.47^{x}
Sunflower hulls	45.21^{A}	48.20^{AB}	49.21^{B}	47.54^{y}
Mean across cycles	48.73^{XY}	47.59^{X}	49.70^{Y}	

^{A,B} Within rows, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{x,y} Across litter types, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{X,Y} Across cycles, means with no common superscripts differ significantly ($P < 0.05$) from each other.

The mean ileum length of broilers at 31 days across treatments indicated that ileum length was the longest ($P < 0.05$) when broilers were reared on PS, the shortest ($P < 0.05$) when broilers were reared on SH and intermediate to the other lengths ($P > 0.05$) when broilers were reared on BS. The mean ileum length of broilers relative to their body weight at 31 days across cycles indicated that broilers reared during Cycle Two had the shortest ileums ($P < 0.05$). The broilers with the longest ileums were found in Cycle Three ($P < 0.05$), and in Cycle One, the ileum length was intermediate to those in the other cycles ($P > 0.05$). Across treatments within cycles, broilers reared on SH during Cycle One had relatively shorter ileums ($P < 0.05$) than broilers on the other treatments. No differences ($P > 0.05$) were reported across treatments in the other cycles. Across cycles within treatments, broilers reared on BS had the shortest ileums ($P < 0.05$) during Cycle Two, the longest ileums in Cycle Three ($P < 0.05$) and in Cycle One the ileum length was intermediate to those in the other cycles ($P > 0.05$). Broilers reared on PS had the shortest ileums ($P < 0.05$) in Cycle Two, the longest ileums in Cycle One ($P < 0.05$), and in Cycle Three, the ileum length was intermediate to those in the other cycles ($P > 0.05$). Broilers reared on SH had the shortest ileums ($P < 0.05$) in Cycle One, the longest ileums in Cycle Three ($P < 0.05$), and in Cycle Two, the ileum length was intermediate when compared to those in the other cycles ($P > 0.05$).

Table 4.8.2.10. Caecum length (cm/kg live weight) of broilers at 31 days

Litter type	Cycle 1	Cycle 2	Cycle 3	Mean across litter types
Biosecure shavings	10.59^{a,A}	9.76^{a,B}	11.08^{a,A}	10.48^{x}
Pine shavings	10.52^{a,AB}	10.20^{a,A}	11.00^{a,B}	10.57^{x}
Sunflower hulls	9.18^{b,A}	10.06^{a,B}	10.12^{b,B}	9.79^{y}
Mean across cycles	10.10^{X}	10.01^{X}	10.73^{Y}	

^{a,b} Within columns, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{A,B} Within rows, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{x,y} Across litter types, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{X,Y} Across cycles, means with no common superscripts differ significantly ($P < 0.05$) from each other.

The mean caecum length of broilers at 31 days across treatments indicated proportionately shorter ($P < 0.05$) caeca on SH. The mean caecum length of broilers across cycles indicated that caecum length was proportionately longer ($P < 0.05$) during Cycle Three, when compared to the other cycles. Across treatments within cycles, broilers on SH had proportionately shorter caeca ($P < 0.05$) than the other treatments during both cycles One and Three. No differences ($P > 0.05$) were found across treatments during Cycle Two. Across cycles within treatments, broilers on BS had shorter caeca ($P < 0.05$) during Cycle Two than in the other cycles. Broilers housed on PS had the shortest caeca ($P < 0.05$) in Cycle Two, the longest caeca in Cycle Three ($P < 0.05$), and in Cycle One, the caecum length was intermediate to those in the other cycles ($P > 0.05$). On SH, broilers had proportionately shorter caeca ($P < 0.05$) during Cycle One when compared to the other cycles.

Table 4.8.2.11. Caecum length (cm/kg live weight) of broilers at 31 days

Litter type	Cycle 1	Cycle 2	Cycle 3	Mean across litter types
Biosecure shavings	10.57^{a,AB}	9.80^{a,A}	11.27^{a,B}	10.55^{x}
Pine shavings	10.54^{a,AB}	10.00^{a,A}	11.11^{a,B}	10.55^{x}
Sunflower hulls	9.28^{b,A}	9.86^{a,AB}	10.36^{a,B}	9.83^{y}
Mean across cycles	10.13^{X}	9.89^{X}	10.92^{Y}	

^{a,b} Within columns, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{A,B} Within rows, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{x,y} Across litter types, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{X,Y} Across cycles, means with no common superscripts differ significantly ($P < 0.05$) from each other.

The mean caecum length of broilers at 31 days across treatments indicated that broilers on SH had, on mean shorter caeca ($P < 0.05$), compared to the other treatments. The mean caecum length of broilers across cycles indicated that caecum length was proportionately longer ($P < 0.05$) during Cycle Three when compared to Cycle One, and significantly longer ($P < 0.0001$) when compared to Cycle Two. Across treatments within cycles, birds provided with SH had shorter caeca ($P < 0.05$) than birds on the other treatments during Cycle One. In the other cycles, no differences ($P > 0.05$) were found across treatments. Across cycles within treatments, on both BS and PS broilers had the shortest caeca ($P < 0.05$) in Cycle Two, the longest caeca in Cycle Three ($P < 0.05$) and in Cycle One the caecum length was intermediate when compared to those in the other cycles ($P > 0.05$). On SH, broilers had the shortest caeca ($P < 0.05$) in Cycle One, the longest caeca in Cycle Three ($P < 0.05$) and in Cycle Two the caecum length was intermediate to those in the other cycles ($P > 0.05$).

Table 4.8.2.12. Gizzard content dry matter (%) of broilers at 31 days

Litter type	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Mean across litter types**
Biosecure shavings	92.36^{a,A}	92.20^{a,A}	93.14^{a,B}	92.57^{x}
Pine shavings	91.99^{b,A}	92.16^{a,A}	93.37^{a,B}	92.51^{x}
Sunflower hulls	91.79^{b,A}	91.95^{a,A}	93.05^{a,B}	92.27^{y}
Mean across cycles	92.05^{X}	92.11^{X}	93.19^{Y}	

^{a,b} Within columns, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{A,B} Within rows, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{x,y} Across litter types, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{X,Y} Across cycles, means with no common superscripts differ significantly ($P < 0.05$) from each other.

The mean gizzard content DM at 31 days across treatments indicated that gizzards of birds on SH had more moisture ($P < 0.05$), compared to the other cycles. The mean gizzard content DM at 31 days across cycles indicated that broiler gizzards in Cycle Three had less moisture ($P < 0.0001$) when compared to the other cycles. Across cycles within litter type, no difference ($P > 0.05$) was seen between cycles with BS, whereas gizzard DM was lower during Cycle One ($P < 0.05$) with both the PS and SH treatments, as compared to the other cycles. Across cycles within litter type, the gizzard DM of broilers on all litter types was significantly higher ($P < 0.0001$) during Cycle Three.

Table 4.8.2.13. Gizzard content acid detergent fibre (%) of broilers at 31 days

Litter type	Cycle 1	Cycle 2	Cycle 3	Mean across litter types
Biosecure shavings	16.02^{A}	11.30^{B}	11.55^{B}	12.96^{x}
Pine shavings	16.74^{A}	12.70^{B}	12.03^{B}	13.83^{xy}
Sunflower hulls	16.79^{A}	13.34^{B}	14.61^{AB}	14.91^{y}
Mean across cycles	16.52^{X}	12.45^{Y}	12.73^{Y}	

^{A,B} Within rows, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{x,y} Across litter types, means with no common superscripts differ significantly ($P < 0.05$) from each other.

^{X,Y} Across cycles, means with no common superscripts differ significantly ($P < 0.05$) from each other.

The mean gizzard content ADF across treatments indicated that ADF was lower ($P < 0.05$) when birds were provided with BS, higher ($P < 0.05$) when birds were provided with SH, and did not differ from the other treatments ($P > 0.05$) when provided with PS. The mean gizzard content ADF in Cycle One was significantly higher ($P < 0.0001$) than in the other cycles. Across treatments within cycles, no differences ($P > 0.05$) were found among any of the treatments. Across cycles within treatments, birds provided with BS and PS had higher gizzard ADF values during Cycle One ($P < 0.05$), when compared to the other cycles. The birds provided with SH had lower gizzard ADF values during Cycle Two ($P < 0.05$), higher values during Cycle One ($P < 0.05$), and no difference ($P > 0.05$) was found in Cycle Three compared to the other cycles.

Table 4.8.3. Summary of bird data parameters at 21 and 31 days in the production cycle

Parameter	**21 days**				**31 days**			
	BS	**PS**	**SH**	**ANOVA**	**BS**	**PS**	**SH**	**ANOVA**
Mean live weight (g) of broilers	931.59^{x}	910.09^{y}	950.81^{z}	**	1764.19^{x}	1765.42^{x}	1816.97^{y}	*
Full proventriculus and gizzard weight (g/kg live weight)	35.53^{x}	36.1^{xy}	37.78^{y}	*	26.90^{xy}	25.56^{x}	27.38^{y}	*
Empty proventriculus and gizzard weight (g/kg live weight)	21.73^{x}	22.57^{y}	23.20^{y}	*	17.51^{x}	16.84^{y}	17.35^{xy}	*
Empty intestinal weight (g/kg live weight)	34.94	29.77	32.33	NS	25.52^{x}	23.80^{y}	23.91^{y}	*
Gizzard length (mm/kg live weight)	51.66	51.23	51.29	NS	31.46	31.35	31.75	NS
Gizzard width (mm/kg live weight)	36.12^{x}	38.56^{y}	37.68^{xy}	*	21.41^{xy}	21.21^{x}	22.22^{y}	*
Proventriculus sphincter width (mm/kg live weight)	13.32^{x}	12.95^{xy}	11.92^{y}	*	8.38^{x}	7.68^{y}	7.48^{y}	*
Duodenum and jejenum length (cm/kg live weight)	97.18^{x}	104.59^{y}	103.72^{y}	*	63.62	64.05	62.50	NS
Ileum length (cm/kg live weight)	73.96	74.93	76.74	NS	49.01^{xy}	49.47^{x}	47.54^{y}	*
Caecum length mean (cm/kg live weight)	15.35^{x}	15.96^{y}	15.4^{y}	*	10.52^{x}	10.56^{x}	9.81^{y}	*
Gizzard content dry matter analysis comparison (%)	92.15^{x}	92.27^{xy}	92.36^{y}	*	92.57^{x}	92.51^{x}	92.27^{y}	*
Gizzard content acid detergent fibre analysis comparison (%)	14.52^{y}	16.87^{y}	18.23^{y}	*	12.96^{x}	13.83^{xy}	14.91^{y}	*

* $P < 0.05$; ** $P < 0.0001$ BS = Biosecure shavings; PS = Pine shavings; SH = Sunflower hulls

^{x,y,z} Across litter types, means with no common superscripts differ significantly ($P < 0.05$) from each other

CHAPTER 5
Discussion

This discussion will focus mainly on the evaluation of three different litter materials, namely pine shavings, bio-secure, fumigated pine shavings and sunflower hulls on their physical characteristics and how this influenced broiler performance parameters.

5.1 Physical characteristics of litter

Litter moisture increased with time over a production cycle, and significant differences were seen among litter types during the earlier parts of the production cycle. The moisture content of SH was significantly less than the other treatments at the initial measurement. Jiménez-Moreno *et al.* (2016) and Kimiaeitalab *et al.* (2017) found that dry mattter (DM) levels of SH were 92.5% and 93% respectively, which is in line with values found in the current study (96%). At seven days, the moisture content of BS was significantly higher than that of PS, and at 14 days SH had more moisture than the other treatments. The final DM measurement at 31 days revealed no difference among litter types.

Litter moisture in other studies varied widely between litter types and poultry houses with moisture values from 15 to 45% (Groot Koerkamp, 1994; Hayes *et al.*, 2000; Miles *et al.*, 2011). Collett (2012) defined wet litter as having more than 25% moisture. In this study, litter moisture percentages (initial DM) varied from around 5% at Day Zero to around 32% at Day 31 in the production cycle (data not shown), therefore only classified as wet by the end of the production cycle. Recent research (Van der Hoeven-Hangoor *et al.*, 2014) has shown that moisture content is a less accurate measure of the amount of water present in the litter, and that water activity is a more useful measurement. It provides information about the fraction of water not bound to solutes in the litter, as well as being closely related to the bacterial load in the litter. Due to financial constraints, water activity could not be measured in the current study.

Low moisture content has a direct financial impact for the producer, due to the relationship between ventilation speeds and litter moisture content. The wetter the litter, the higher the ventilation speed required to dry it and the higher the cost (Dunlop *et al.*, 2015). Some studies (Allain *et al.*, 2009; Youssef *et al.*, 2010; De Jong *et al.*, 2014) found a link between the FPD incidence and litter moisture, but no such link was found in the current study, concurring with the results of Škrbić *et al.* (2015).

The water-holding capacity (WHC) of BS and PS declined steadily over the production cycles, but SH increased in WHC. The WHC was measured at several intervals during the cycle and differences were found among litter types at the initial measurement, seven days and 14 days, when SH had a significantly lower WHC as compared to the other litter types. The initial WHC values of the litter treatments in this study correspond to other studies (Garcês *et al.*, 2013; Jiménez-Moreno *et al.*, 2016). The litter types converged towards similar WHC, as no statistical differences were found at the end of the production cycle.

No clear correlation could be seen between WHC and gizzard size in this study. However, Svihus *et al.* (2002) found a correlation between WHC and gizzard size. Fibre sources with a high WHC led to an increase in bulk of gizzard digesta and, therefore, gizzard size. Dunlop *et al.* (2015) found that WHC of litter increased as the production cycle progressed. A reason for this may be due to differences in methods, as litter in the current study was dried prior to testing WHC and a constant litter weight was used across the production cycle. Garcês *et al.* (2013) found that WHC (on a DM basis as in this trial) of several alternate litter materials to PS could become greater or lesser, depending on the litter type. It was also hypothesised (Dunlop *et al.*, 2015) that the true WHC of poultry litter would be a value in between that of compacted litter and litter allowed to settle under its own weight (expressed as the volume of water contained in one m^2; L/m^3 in the mentioned study). This happened because the chickens would scratch and loosen some of the litter while certain parts, for instance around feeders, may be compacted.

Large differences were found between cycles in the water-releasing capacity (WRC) of litter. The results could not be used to draw any conclusions.

Litter caking was assessed at days 21 and 31 of the production cycles. At 21 days the highest caking score was found for BS and the lowest for PS but this effect diminished at 31 days. Garcia *et al.* (2012b) also found differences at 28 and 35 days of a 42-day grow-out, however, the highest caking score was on PS and the lowest on rice husks. Other studies (Bilgili *et al.*, 2009; Garcia *et al.*, 2012b) found correlations between footpad dermatitis (FPD) and litter caking, and although differences in the current study were not significant, broilers did have numerically corresponding FPD scores when compared to litter caking levels at Day 21. Litter propensity to caking was dependent on particle size as well as litter type. Litter containing larger particles (> 2.5 cm) tended to clump together more readily (Grimes *et al.*, 2002). In the current study, BS had the largest particles (~ 2 cm), which may explain the increased caking score at the 21-day measurement.

The bulk density (BD) of the litter was also examined in this study and it increased as the production cycle progressed in accordance with Garcês *et al.* (2013). The change in BD between the initial and final measurements was the smallest for SH, and the largest for BS. In the current study, litter BD increased by 2.1 times on SH, 3.2 times on PS and 7.5 times on BS. In a study by Garcês *et al.* (2013), litter BD increased on average 2.4 times. The initial BD of litter differed across all three litter types so that BS had the lowest BD, followed by PS, and the highest BD was observed for SH. This difference among litter types continued at seven days, but at 14 days, there was no statistical difference between PS and SH. By 21 days, no difference was seen between litter types for the remainder of the production cycle. In a study by Bilgili *et al.* (2009) testing alternative litter sources to PS, the litter type with the highest initial BD was also found to have the lowest WHC and lowest moisture level, which was a similar result to SH in this study. In a recent review by Dunlop *et al.* (2016), BD was found not to be one of the critical factors affecting litter susceptibility to wetness.

The litter pH increased at similar linear rates on all treatments as the production cycle progressed. At seven days, there were significant differences between BS and PS, with PS having the lowest pH (5.99), and BS the highest (6.11). Litter that initially had a lower pH had better ability to prevent uric acid conversion to ammonia than litter with high initial pH (Moore *et al.,* 1996). At 14 days, PS had the highest pH and SH had the lowest pH. These differences diminished after 14 days, and no further differences could be found in pH among litter types. The lack of further differences in pH was due to the accumulation of feed, faeces and water in the different treatments (Davasgaium & Boodoo, 1997; Garcês *et al.*, 2013). The pH at the conclusion of the trial (8.2-8.5) was similar to results found in several studies (Moore *et al.,* 1996; Terzich *et al.*, 2000; Garcês *et al.*, 2013). According to a report by Terzich *et al.* (2000), the average pH across 12 poultry producing areas in the USA was 8.0.

5.2 Proximate analysis of the litter

Litter moisture was discussed as part of the physical characteristics, as it ties in well with those parameters. All further lab analyses were done on a dry matter basis.

The litter CP increased linearly in all three litter types as the production cycle progressed. For BS and SH, quadratic equations also fit the model. Upon investigation into whether differences existed among litter types during the course of the cycle, it was found that initially, SH had a significantly higher CP value than the other treatments. The CP in the litter was analysed at seven days, and no difference between BS and SH was recorded, but PS had a significantly lower CP level. At 14 days, BS had a significantly higher CP level than the other treatments, but the differences between treatments diminished by the end of the cycle.

The initial CP measurements showed discrepancies between SH and the other treatments since SH had a much higher CP value ($P < 0.0001$) of 6.36% versus 0.84% and 0.88% for BS and PS, respectively. The initial CP values of BS and PS were in line with litter materials used in other studies (Atapattu & Wickramasinghe, 2007; Garcês *et al.*, 2013). Kimiaeitalab *et al.* (2017) found that CP levels of SH (% as fed) in diets of broilers was 4.7%, but in the study of Jiménez-Moreno *et al.* (2016), the CP level was 6.3%, which is in line with the level found in the current study.

The high CP values reported at the end of the experiment may favour the litter to be utilised as organic fertiliser or ruminant feed, as suggested in Atapattu & Wickramasinghe (2007) and Garcês *et al.* (2013). Litter CP was not a true representation of the amount of nitrogen present in the litter as nitrogen was continually lost to the environment in the form of ammonia, especially with high temperatures and humidity (Atapattu & Wickramasinghe, 2007; Garcês *et al.*, 2013).

Similar trends were seen in the initial EE values with SH having a value of 8.49% versus 0.64% and 1.06% in BS and PS, respectively. The initial ash value was also significantly higher ($P < 0.05$) in SH as compared to the other treatments. Other studies found that EE levels of SH (% as fed) of broilers was 4.6% and 3.6%, respectively (Jiménez-Moreno *et al.*, 2016; Kimiaeitalab *et al.*, 2017). The same authors found that ash levels of SH were 3.6% and 2.8%, respectively.

The initial ADF values of all three litter types differed significantly from each other, and SH had an ADF value that was 18 and 20% lower than PS and BS, respectively. Jiménez-Moreno *et al.* (2016) and Kimiaeitalab *et al.* (2017) found that ADF levels of SH (% as fed) of broilers were 46.7% and 51.3% respectively, lower than 63.5% ADF found in SH in the current study.

The analyses were repeated at the end of the production cycles and results of EE and ash analyses indicated no differences among treatments. The ADF values indicated differences between BS and PS, and the ADF level across treatments dropped significantly when compared to the initial analyses. As evident from other litter physical characteristics in this study, litter types become more similar as the production cycle progresses with more faeces, feed and feathers added to the litter, thereby homogenising it (Davasgaium & Boodoo, 1997; Garcês *et al.*, 2013).

5.3 Litter bacteria

No *Salmonella* bacteria were found on the litter at any point in the study. The *Salmonella* boot swabs yielded no positive results throughout the study. It can thus be ruled out as a contributing factor to wet litter in all the houses. It also confirms that the farm had sufficient biosecurity measures in place to prevent a *Salmonella* outbreak, and adhered to biosecurity measures recommended by the European Food Safety Authority (2004).

Bacterial tests for *E. coli* and *Salmonella* were conducted on the intestines of broilers but were abandoned due to the large individual variation in bacterial populations between broilers. Torok *et al.* (2009a) reported that large individual variations exist between immature broilers and between sections of intestines, because the gut microbiome is not yet established.

5.4 Effect of litter on footpad dermatitis

The FPD scores of broilers were not affected by litter type in this study, in agreement with Sorbara *et al.* (2000) and Teixiera *et al.* (2015). In contrast to this, broilers housed on wood shavings versus chopped straw were found to have a lower incidence of FPD (Skrbic *et al.*, 2015), but straw has been found to be abrasive and a less favourable litter source (Bilgili *et al.*, 2009; Skrbic *et al.*, 2015). Several studies have shown that FPD is affected to a larger extent by litter management factors such as litter moisture, ammonia levels, particle size and stocking density (Bilgili *et al.*, 2009; Cengiz *et al.*, 2011; Zhang *et al.*, 2011; Garcia *et al.*, 2012b; De Jong *et al.*, 2014). The FPD scoring was done on two occasions during the production cycle, at 21 and 31 days, respectively. The severity of FPD increased as the broilers became older since their rapidly increasing weight put more pressure on their footpads. Martland (1985) and more recently Tiara *et al.* (2014) reported that birds that were housed on wet litter and then transferred to dry litter showed vastly improved FPD lesion scores after one week. After two weeks, the lesion scores were indistinguishable from the control group in the Martland (1985) study. A turnaround strategy to reduce the FPD incidence before the birds were sent for slaughter would be difficult to implement because the production cycle is so short.

FPD is not only a welfare issue, but also presents itself as an economic burden on producers (Shepherd & Fairchild, 2010), since prices of chicken feet have been increasing locally, as well as for the export market to Asian countries. It is important for producers to focus on controlling FPD in order to increase revenue. In a study by Menzies *et al.* (1998), which compared 950 commercial broiler flocks, the incidence of hock burn was decreased on farms where producers achieved production targets. This points to the multi-factorial nature of FPD and that management plays a large role in its occurrence.

5.5 Production parameters

Across litter types, broilers on SH yielded the highest kg/m^2 as well as achieving the highest average slaughter weight, ADG and lowest seven-day mortality. Jiménez-Moreno *et al.* (2016) found that broilers improved their ADG by 2.1% when 5% SH was included in their diets. Various studies did not find differences in ADG among several other litter types, such as PS, coir dust, rice husks and refused tea (Swain & Sundaram, 2000; Atapattu & Wickramasinghe, 2007; Cengiz *et al.*, 2011).

No differences were found in total feed consumed, FCR, PEF or 33-day mortalities among litter types in this study. Numerous other studies found no differences among parameters such as BW, FCR (Brake *et al.*, 1992; Torok *et al.*, 2009a), mortalities (Toghyani *et al.*, 2010; Teixiera *et al.*, 2015), and total feed consumption (Swain & Sundaram, 2000). Samarakoon & Samarasinghe (2012) found that PEF is still a robust measure of production evaluation, even though other production equations have recently been suggested. In their study, a change of 15 PEF points led to significant differences in production margins. Small changes were not statistically significant, but may be economically significant, although a cost analysis would need to be done.

5.6 Broiler gut development parameters

5.6.1 Litter effects on body weight

The 21-day data of birds selected for gut development tests showed significant differences across litter types. Broilers reared on SH had the heaviest 21-day weight and broilers reared on PS had the lowest body weight (BW) as compared to the other treatments. This phenomenon continued in the 31-day data, where broilers on SH still had heavier BW when compared to the other litter types. This parameter was consistent with the production parameters at 33 days, where the average slaughter weight of all birds per treatment was measured and birds reared on SH had the highest weight. Toghyani *et al.* (2010) also found significant differences in live weight when comparing PS, sand, rice husks and paper roll with broilers reared on rice husks exhibiting the lowest BW ($P < 0.05$) at 42 days of age. A possible explanation for the heavier BW on SH points to the higher EE and CP levels ($P < 0.05$) of the SH during the earlier parts (initial measurements) of the production cycle. Thus, when the broilers consumed the SH, they received more nutritional compounds when compared to broilers consuming the other litter types. The growth of broilers up to 21 days of age increased when structural fibre, namely SH, oat hulls or rice hulls were added to their diets at 2.5% and 5% (Jiménez-Moreno *et al.*, 2016). Fibre added to the diet of broilers in the form of SH had no adverse effect on broilers when the fibre percentage of diets containing SH had 50% more fibre than control diets. Insoluble fibre added at these levels served as an inactive diluent of the diet (Viveros *et al.*, 2009).

All further measurements were made as a proportion of BW in order to reduce individual variation and effect of BW on the measurements. The weight and length of organs all declined relative to BW at 31 days as compared to 21 days, since the broilers gain more muscle mass in the latter part of the production cycle. The absolute weight and length of all organs increased from 21 to 31 days. These findings are consistent with González-Alvarado *et al.* (2008), who also measured organ weight and length as a percentage of BW.

5.6.2 Litter effects on gizzard development and content

It is well-established in the broader literature that providing broilers with coarse particles in the diet increases gizzard size (Hetland *et al.*, 2004; Amerah *et al.*, 2008; Mateos *et al.*, 2012; Sacranie *et al.*, 2012). In this study, the coarse particles came from the litter. During dissection, litter material was observed as part of the gizzard content. The gizzard ADF content was much higher than in feed, indicating that broilers did consume their litter, a finding that is in agreement with Hetland *et al.* (2003). The ADF content in the feed was between 5.6-7.8% on the different feeding phases, whereas the ADF content in the gizzards was between 13-18.2% on the various litter types. On closer inspection of the ADF differences between litter types, it was noted that the ranking of ADF content between litter types did not change across time, with broilers reared on BS having the lowest ADF content (14.5% and 13%) at 21 and 31 days, respectively. Broilers reared on PS had significantly higher gizzard ADF than BS at 21 days, but not at 31 days, however, the ADF content was numerically higher (16.8% at 21 days and 13.8% at 31 days). On both days, the ADF content of SH was significantly higher than BS (18.2% at 21 days and 14.9% at 31 days). These values may suggest that broilers consumed more SH than the other litter types. From production parameters, broilers reared on SH also had heavier BW when compared to the other treatments as discussed earlier. When broilers consumed coarse particles, the particles remain in the gizzard for a longer period, until it was ground fine enough to pass into the duodenum. This increased amount of gizzard contractions, improved gizzard musculature and reduced the pH of gizzard digesta, which ultimately improved gut development (Hetland *et al.*, 2003; Kimiaeitalab *et al.*, 2017).

The gizzard content of broilers reared on BS had significantly more moisture when compared with gizzard content from broilers reared on SH at day 21 of the production cycle. At day 31, gizzard digesta had more moisture when broilers were reared on SH than on the other treatments. Jiménez-Moreno *et al.* (2016) found that including 5% SH or rice hulls in diets increased the moisture content of digesta due to the higher WHC of the insoluble fibre sources when compared to oat hulls. No differences in WHC were observed among litter types at Day 31 of the production cycle in the current study.

The empty proventriculus and gizzard weight of broilers at 21 days differed between BS and the other treatments. Broilers reared on PS and SH had relatively heavier proventriculi and gizzards than those on BS. On day 31, broilers on BS had the heaviest proventriculi and gizzards, broilers on PS had the lightest proventriculi and gizzards and intermediate weights on SH. Kimiaeitalab *et al.* (2017) found that broilers had heavier gizzards when 3% SH was included in their diets at 21 days. Birds consuming hard, large particles of insoluble fibre (oat hulls) were found to have the heaviest gizzards, as well as birds that had access to wood shavings instead of being caged (Sacranie *et al.*, 2012).

However, no gizzard weight difference was found between PS and refused tea in a study by Atapattu & Wickramasinghe (2007).

The gizzard dimensions were also of importance since statistical differences existed between gizzard width and proventriculus sphincter width. At 21 days, broilers reared on PS exhibited the widest gizzards and the narrowest were found in broilers reared on BS. At 31 days, the picture seemed reversed, when the narrowest gizzards were recorded among broilers reared on PS, and the widest on SH. The gizzard width correlated mostly with the results found on gizzard weight. The proventriculus sphincter width was the widest when broilers were reared on BS at both 21 and 31 days, and the narrowest on SH at 21 days. No significant differences were found between SH and PS at 31 days. No significant differences were found in gizzard dimensions on pelleted versus mashed diets at 21 days of age (Amerah *et al.*, 2007). However, Hetland *et al.* (2004) found that gizzard dimensions increased when coarse particles were retained in the gizzard.

5.6.3 Litter effects on intestinal weight and length

The relative duodenum and jejunum length (cm/kg BW) of sacrificed broilers revealed significantly shorter intestinal lengths at 21 days when broilers were reared on BS, as compared to the other treatments, but this discrepancy disappeared at 31 days. Ileum length did not differ at 21 days, but at 31 days, broilers on SH had significantly shorter ileums than broilers on PS. Caecum length was longer in broilers reared on PS at 21 days, and shorter in broilers reared on SH at 31 days, as compared to the other treatments. Caecum length in broilers can increase significantly after five weeks on a higher fibre diet (barley diet versus an oat-based diet) according to Jozefiak *et al.* (2006). However, Kimiaeitalab (2017) found that neither absolute nor relative intestinal tract length was affected by the inclusion of 3% SH in the diets of broilers during the first 21 days of the production cycle.

The relative intestinal weight showed no differences at 21 days, but was heavier in broilers reared on BS at 31 days than with the other litter types. No differences were found in intestinal length among different fibre sources for broilers up to 22 days old (González-Alvarado *et al.*, 2008)

The type of fibre source provided to broilers will influence the broilers' response to the fibre, since fibre sources differ in several properties, such as WHC, particle size and fibre percentage (Bach Knudsen, 2001; Svihus et al., 2002; González-Alvarado *et al.*, 2008). Several studies have concluded that the inclusion of moderate amounts of fibre (2.5-5%) improved broiler performance from a gut development point of view, but that this effect was reversed when amount was higher than 10% (Hetland *et al.*, 2003; González-Alvarado *et al.*, 2007; Mateos, 2012; Jiménez-Moreno *et al.*, 2016).

5.7 Litter beetle activity on the different litter types

The propensity of the different litter types to litter beetle infestation was investigated by looking at the incidence of *A. diaperinus* at each stage of the life cycle weekly. When comparing across litter types over the three cycles, the most worms and beetles were found on PS, which was also the litter type with the highest overall beetle activity. The lowest overall beetle activity was found on SH, but another species of beetle was also found on this litter type (mentioned below). The least worms occurred with the use of SH, whereas the least beetles occurred on BS. Comparable research into the effect of different litter types on beetle infestation in broiler houses is lacking. The level of beetle activity increased with progression of the production cycles in agreement with Stafford *et al.* (1988).

When comparing trap positions in the houses, *A. diaperinus* preferred sites in the half of the house that was used for brooding during the first four days of production. Previous research (Strother & Steelman, 2001; Chernaki-Leffer *et al.*, 2007) have aimed to devise models for the spatial arrangement of litter beetles, and have found that litter beetles do not necessarily aggregate in areas most suitable for their survival, but in the longer term beetles do conform to the model. The model dictated that beetles would aggregate near feed and water lines, as well as close to the walls. *A. diaperinus* in this study did conform to this model, but traps were also placed in locations predicted by the model and other recommendations (Safrit & Axtell, 1984; Stafford *et al.*, 1988).

The different preferences in positions of the various life cycles indicated differences in needs of the beetle. The worms may have been more set on finding nutrition from the hopper, as feed spillage was more likely to occur there, pupae needed shelter under the motor, where there were crevices to pupate and adults emerged from the shelter in the cracks of the walls of the house, where movement to various areas can occur and tunnels were used for oviposition (Axtell & Arends, 1990). Even though adults were found near the walls of the house, this was mainly due to utilisation of the insulation for pupation. Geden & Axtell (1987) found that beetles tunnelled more readily in walls of the broiler house when beetle density was increased, and when litter material was lacking. Most climbing occurred during dark periods. Similar behaviour was observed for larvae in experimental conditions (Geden & Axtell, 1987).

Tribolium castaneum, of the family Tenebrionidae (red flour beetle), was found only on the SH throughout the trial. Since the houses were rotated with each production cycle, it is clear that these beetles were an effect of the litter and not of the houses. None of these beetles were found in other houses that contained SH in previous production cycles. These beetles are 2.4-4.2 mm in length and have shiny, reddish-brown elongate bodies. They occur in milled products as well as in a variety of oilseeds (Kruger, 2016). Damaged sunflower seeds and SH were preferred substrates to other seeds (canola, flaxseed) for these beetles to infest (White & Jayas, 1993). Damaged seeds provide nutrition for the beetles, while the hulls provide shelter and protection. The beetles tended to survive best in seed fractions with the highest nutritional value, and they preferred the seed kernel to the hulls. In the study by White & Jayas (1993), *T. castaneum* survived in stored SH as well as other forms of the sunflower seed (ground, whole and de-hulled) for two months. Further research would be required to investigate whether the presence of *T. castaneum* has a negative impact on the presence of *A. diaperinus* due to the lower infestation of *A. diaperinus* on SH.

CHAPTER 6

Conclusion

Broilers that have access to litter materials do consume their litter, which was evident in the increased ADF levels found in gizzard contents versus feed. This led to improved gut development in the case of SH, which translated to improved ADG, kg/m^2 and slaughter weight. The SH contained more nutrients based on proximate analysis, as compared to the other litter types. However, improvements seen with SH did not alter the commercially measured figures of PEF and FCR. The SH carried a litter pest (*T. castaneum*) not often associated with poultry houses, which could hold as yet unidentified disadvantages to producers. The SH had the lowest overall *A. diaperinus* activity, which may offset disadvantages. Biosecure pine shavings had no superior effect when compared to PS. Litter converge toward similar physical characteristics at the end of a production cycle, due to addition of feed, feathers and excreta. Management of litter remains an important part of achieving production targets, irrespective of the litter type used.

CHAPTER 7

Critical Review

Challenges were added to the study due to the commercial scale of the project, which made it difficult to keep the constants similar. The feed formulation and litter availability from the same suppliers were especially challenging, since the country was going through a crippling drought at the time of the study. The project also faced several financial challenges.

The WRC should have been measured using a more precise method. The results received could not be used to draw conclusions. Individual variation would have been overcome if a larger sample size were used to monitor intestinal bacterial levels of broilers. Cycle differences were seen for several parameters, which would have been prevented if the study ran simultaneously, however this would have been impractical on a commercial scale.

There were a few parameters that would have improved the study if they were included, but it was not economically feasible. Water activity has recently been established as a good measure of the amount of free water in litter, and has been closely correlated with litter bacterial levels. Ammonia levels are closely associated with FPD incidence and respiratory health of broilers, and could have influenced the CP levels measured in the study. There was also evidence of high ammonia levels at one point in the study, which was confirmed by the farm veterinarian, but it could not be included as part of the study.

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