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Litter characteristics of pine shavings, bio-secure pine shavings and sunflower hulls and its impact on broiler performance

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Abstract

The aim of this study was to investigate the physical characteristics of three litter materials, namely pine shavings (PS), bio-secure, fumigated pine shavings (BS) and sunflower hulls (SH) and its influence on broiler performance over a 33-day production cycle. The experiment was conducted in commercial poultry houses holding 42,500 chicks each, utilising a randomised block design with six house replicates per treatment. Litter samples were collected weekly for analyses of moisture, water-holding capacity, bulk density, pH and litter caking. Broiler footpad dermatitis was monitored at 21 and 31 days, together with acid detergent fibre (ADF) concentration of gizzard content, gizzard weight and small intestinal weight and length of 120 birds per treatment. Broilers across treatments consumed litter material which was evident in increased ADF levels of gizzard contents relative to feed. The SH contained more nutrients based on proximate analysis as compared to other treatments. Rearing on SH led to lower 7-day cumulative mortality, higher kilograms of broilers produced per square meter, average daily gain and slaughter weight. Improvements seen with SH did not alter commercial indicators, namely, production efficiency factor and feed conversion ratio. Litter converged toward similar physical characteristics at the end of production cycles when few differences were observed between treatments due to addition of feed, feathers and excreta.

Keywords: Bedding material, intestinal weight, litter physical characteristics, poultry production

1 Introduction

The South African broiler industry represents around 1.8 % of the global market and is the country's largest agricultural sector, as well as the largest producer of poultry in Africa (Nkukwana, 2019). The South African industry is structured similarly to the global industry with large-scale integrations and contract growers, but also includes a large amount of smaller scale producers. Through rural development programs, many small-scale farmers have been able to produce for the informal and even formal market. However, economy of scale is a large barrier of entry for small-scale producers (Louw *et al.*, 2010). South Africa struggles to maintain global competitiveness, due to growing importation of dark meat below production costs, high input costs, as well as a delay in land allocation for small-scale producers to increase their production (Nkukwana, 2018). In the South African broiler industry, bedding, waste removal and cleaning comprises 1.7 % of the variable costs of operation per production cycle (Davids & Meyer, 2017).

The main drivers of monetary return in poultry production rest on weight gain, feed conversion, and mortality rate. Commercial enterprises tend to focus on chick and feed quality while bedding material and litter quality are often neglected (Xu *et al.*, 2017). However, several studies have indicated that achievement of live production targets depend on bedding source and litter quality (Ritz *et al.*, 2009; Torok *et al.*, 2009; Garcês *et al.*, 2013, Kheravii *et al.*, 2017a), which could ultimately affect the profitability of broiler production.

Poultry litter consists of the substrate utilised for bedding material mixed together with bodily excretions, feathers as well as spilt feed and water (Ritz *et al.*, 2009). Selection of bedding material mostly depends on availability within the area. In South Africa, commonly used bedding materials for poultry production include wheat straw, sawdust, wood shavings, sunflower hulls and peanut hulls (Jordaan, 2004). Ideal bedding sources include pine shavings and pine sawdust, but these have become scarce and increasingly expens-

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ive (Awojobi *et al.*, 2016). Sunflower hulls as a bedding source are inexpensive, seasonally available and widely used in South Africa, however, limited research has been done into its viability as an alternate bedding source (Jordaan, 2004).

Several studies did not find any differences in broiler performance when utilising re-used litter in experimental conditions (Bilgili *et al.*, 2009, Torok *et al.*, 2009; Toghyani *et al.*, 2010, Garcia *et al.*, 2012a). However, re-using of litter is rarely practiced in South Africa and comparatively little research has been done on complete clean out of litter at the end of each cycle under commercial conditions. Optimal broiler performance and welfare is dependent upon reliable suppliers of high quality bedding (Grimes *et al.*, 2006). Bulk density is an indirect indication of porosity, water-holding capacity and water-releasing capacity of litter (Atapattu & Wickramasinghe, 2007). Water-holding capacity and evaporation capacity of litter is reduced when litter caking occurs (Collett, 2012; Dunlop *et al.*, 2015). Litter holding more than 65 % moisture promotes the growth of bacteria and moulds through fermentation of the litter (Ritz *et al.*, 2009). Higher quantities of environmental bacteria were found on fresh litter as opposed to re-used litter and litter conditions such as pH and moisture affect the type of bacteria present in litter (Cresmann *et al.*, 2010). Pathogenic bacteria increase as pH becomes more alkaline (Wang *et al.*, 2016). Litter pH is generally alkaline and tends to increase in alkalinity as excreta accumulate over the production cycle (Garcês *et al.*, 2013). The ammonia to ammonium ratio is determined by litter pH and conversion to ammonia occurs at high pH, with increasing volatilisation as the pH increases (Miles *et al.*, 2011).

The bedding source used in a broiler house can influence gizzard development and digestive function (Xu *et al.* 2017). Malone *et al.* (1983) found that broilers could consume up to 4 % of their diet in the form of litter material. Ingesting of coarse materials, such as pine shavings, benefit gizzard development (Amerah *et al.*, 2008; González-Alvarado *et al.*, 2008), which could aid in better digestion and feed conversion (Mateos *et al.*, 2012). Muscular hypertrophy of the gizzard contributes to more frequent reverse peristalsis, which can alter intestinal microbial composition, potentially enhancing nutrient availability to the chicken. Microbial competitive exclusion also occurs which reduces pathogenic bacterial loads and ultimately improves gut morphology (Torok *et al.*, 2011; Wang *et al.*, 2016).

Broiler welfare is influenced by the type of bedding in the house and resultant litter conditions. Footpad dermatitis (FPD) is considered as a health implication of poor welfare and litter conditions (Shepherd & Fairchild, 2010). Footpad dermatitis, which occurs as burns on the feet of broilers, can develop in a few days when litter conditions are suboptimal and severe lesions tend to occur early in the growing period (Hoffmann *et al.*, 2013) due to the constant contact between the footpads of the birds and the litter.

The aim of the study was to evaluate the physical characteristics of three different bedding sources and whether this has an effect on performance, mortality, FPD and intestinal weight and length of broilers under commercial conditions. Three types of commonly used bedding materials in South Africa were investigated in this project, namely bio-secure, virgin pine shavings, non-chemically treated pine shavings, and sunflower hulls.

2 Materials and methods

All animal care procedures were approved by the University of Pretoria's Animal Ethics Committee (Project number EC 048-15). Six identical, fully environmentally controlled broiler houses near Ogies in the province of Mpumalanga (co-ordinates 26°03'53.5"S, 28°50'33.6"E) were used in the study. The houses were controlled by an electronic climate control system (ViperTouch, Big Dutchman South Africa, Johannesburg). The houses were $123\,m \times 15\,m \times 2.4\,m$ (foundation to roofline) in dimension. Housing management conditions were in accordance with recommendations by the Ross Broiler Management Guide (2014). The stocking density was approximately 42.8 $kg\,m^{-2}$ (23 $birds\,m^{-2}$) for each cycle and all houses were initially stocked with 42,500 chicks per house. Final sampling was done on day 31 to allow birds to recover from stress due to sampling before slaughter, and only slaughter parameters were measured at 33 days. The three bedding types used in the trial were (1) bio-secure, fumigated virgin pine shavings (BS); (2) non-chemically treated pine shavings (PS) and (3) sunflower hulls (SH). Bio-secure shavings were virgin pine shavings cut to $2\,cm^2$, low-dust and fumigated prior to packaging in sealed plastic bags to ensure minimum contamination with pests and bacteria. Particle size of PS was approximately $1\,cm^2$ and SH $1\,cm \times 0.4\,cm \times 0.3\,cm$. Within each production cycle, three litter types were randomly allocated to six houses, thus each litter type was replicated twice per cycle. A different randomisation was utilised for each production cycle. By the end of the experimental period, each of the three bedding treatments were replicated six times with a total number of 255,000 birds per treatment as part of the trial. All data collected within a house was averaged and analysed as an experimental unit.

The trial was run in three non-consecutive production cycles of 33 days each and complete cleanout of litter was practiced between production cycles to prevent any carry-over effect. Each bedding material was received from the same supplier throughout the trial. Before chick placement at the start of each new production cycle, bedding material was evenly spread over the concrete floors of the houses with a roller to an approximate depth of 50 mm. Topdressing was only done in areas where drinker nipples leaked or where caking scores reached 100 % and the wet litter was becoming a health hazard to the broilers.

Litter samples were collected at three areas in the house - in opposite corners and in the centre. All the litter in a 0.5 m^2 area was removed, mixed well, and subsamples were analysed for several parameters.

Representative samples of fresh bedding material, as well as litter on day 31 was analysed for dry matter (DM; AOAC, 2000; method number 934.01), ash (AOAC, 2000; method 942.05), crude protein (CP; Leco-Dumas method 968.06), acid detergent fibre (ADF; Ankom Technology Method 8, filter bag technique for A2000) and ether extract (EE; AOAC, 2000; method 920.39). Weekly litter samples were collected and analysed for bulk density, water-holding capacity, litter pH and litter moisture, which was calculated by subtracting the DM of the litter from 100. Bulk density was measured as the mass of litter (on 'as is' basis) that fit in a 1 L beaker (Garcês *et al.*, 2013).

Water-holding capacity was determined as follows: each litter sample was dried at 55 °C until constant weight and 50 g placed in a 500 mL beaker; the beaker was filled with water and left to stand for 30 minutes; excess water was drained for 3 minutes through a 850 µm sieve; and then weighed again, where after percentage water absorbed could be calculated (Brake *et al.*, 1992; Garcês *et al.*, 2013).

Litter pH was determined by suspending a macerated 30 g litter sample in 250 mL 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). Litter caking was scored at 21 and 31 days. A 50 cm^2 frame was placed at five areas in the house - in each corner and in the centre of the house. The frame was flipped four times to form 1 m^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 = 1/4 of the square caked;

2 = 1/2 of the square caked;

3 = 3/4 of square caked; and

4 = whole square caked.

These values were then averaged per house and converted to percentages.

Mixed-sex day-old Ross 308 chicks were received from the same hatchery, and parent flock ages were recorded. Where possible, offspring from different aged parent flocks were evenly distributed, such that over the three cycles, each bedding treatment received a similar distribution in chicks from different parent flock ages. Half-house brooding was utilized for the first four days of production. Broilers were fed commercial broiler feed with a pre-starter diet for days 0–7, a starter diet from days 8–14, a grower diet from days 15–21, a finisher diet from days 22–28 and a post-finisher diet from days 29–32. As soon as feed was dispensed, one sample per week of the feed was taken from the central hopper of each house, and pooled together. Representative samples of the pre-starter, starter, grower, finisher and post-finisher feeds were collected in this manner. Feed was analysed for DM, ash, CP, ADF and EE using the same methods as for the litter.

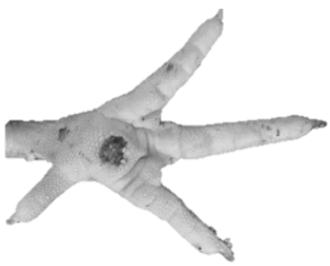
Broiler body weight, feed and water intake of birds were monitored daily per house replicate using automated flowmeters and scales (Swing 20, Big Dutchman SA). Broiler mortality was recorded daily and cumulative mortality to seven and to 33 days was analysed. Each production cycle was terminated when the broilers reached 33 days of age and mean values of several production parameters were analysed from data received on this day. Total feed consumed per house replicate, kilograms of broilers produced per square meter, mean broiler live weight at slaughter (in kg), commercial feed conversion ratio (FCR), average daily gain (ADG) and production efficiency factor (PEF) were calculated on this day (after Marcu *et al.*, 2013).

$$\text{PEF} = \frac{\text{body weight (kg)} \times \text{livability(\%)}}{\text{slaughter age (days)} \times \text{FCR}}$$

At 21 and 31 days of age, 20 broilers (10 males and 10 females) per house replicate, and therefore 120 birds per treatment, were randomly selected from both the front and back areas of the houses. They were sacrificed via cervical dislocation and were weighed in grams (LBK 3, Adam Equipment South Africa, Johannesburg) before evisceration.

The digestive tract was excised from the proventriculus to the cloaca and mesentery and fat removed from the organs. The gizzard and proventriculus were removed from the intestines at the proximal end of the duodenum. The gizzard was opened, contents of two birds per house replicate randomly pooled to ensure a large enough sample size for ADF determination (thus 60 observations per treatment) and analysed for DM (AOAC, 2000; method 934.01) and ADF (Ankom Technology Method 8, filter bag technique

Table 1: *Footpad dermatitis (FPD) scoring method utilised during the trial*

Class	*Picture*	*Description*
1		Footpad exhibits no external signs of FPD, footpad is soft to the touch, no redness evident on footpad.
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		Lesion covers up to half of the footpad, hyperkeratosis is seen around the lesion, marked swelling on footpad.
5		As for Score 4, but lesion covers more than half of the footpad, litter may stick to the footpad due to oozing of the lesion.

for A2000); ADF was expressed on DM basis. The empty proventriculus and gizzard were weighed in grams (PB303-SRS, Mettler Toledo) according to procedures described by Starck (1999).

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 (duodenum and jejunum length combined) and from Merkel's diverticulum to the ileocaecal junction (ileum length). Following measurement, the intestinal contents were milked out and the empty intestines were weighed. The weight and length of the intestines were expressed relative to the weight of the bird in order to reduce variation.

Footpads of broilers were examined and scored for FPD in a similar fashion to the European system for scoring FPD in turkeys (Hocking *et al.*, 2008). Footpad scoring is considered the most sensitive indicator of pododermatitis and is preferred above examining hock or breast blisters. Forty randomly selected birds from four locations per broiler house replicate were scored at 21 and 31 days of age; thus 240 birds per treatment were scored at 21 days and 240 birds per treatment at 31 days. Birds were picked up, footpads were scored and birds were placed in a makeshift pen until scoring in the area was completed, before being released. Scoring was conducted as depicted in Table 1.

The experimental design was a randomised block with six replications (houses). Data was analysed using SAS version 9.2 (SAS Institute, 2013). Analysis of variance (ANOVA) was conducted using the GLM model to test for a significant bedding source (treatment) effect. For repeated measurements over time within production cycles, time was included as a sub-plot factor in the ANOVA and the interaction between day and litter was also tested. The Shapiro-Wilk test was used to test for normality. Where applicable, Pearson correlation coefficients were utilised. A p-value of less than 0.05 was considered statistically significant. Means of significant effects were separated using Fishers' protected t-test least significant difference (LSD).

3 Results

Table 2 shows the mean nutrient content (CP, EE, ash and ADF) of the fresh bedding material (day 0) and of the litter material at conclusion (day 31) of the production cycles. At the commencement of the production cycles, the mean SH contained higher levels ($p < 0.05$) of CP, EE and ash and lower ($p < 0.05$) ADF than both BS and PS. BS had a slight but significant higher ADF and lower EE than PS. At the conclusion of the production cycles, the mean CP, EE, ash and ADF did not differ ($p > 0.05$) between treatments.

Table 3 summarises the litter moisture percentage, water-holding capacity, bulk density, pH and caking of the three litter types at various intervals across the production cycles. Litter moisture percentage indicated no significant differences between the treatments from days 0 to 21 ($p > 0.05$). At day 31, SH contained ($p < 0.05$) less moisture than both BS and PS.

Sunflower hulls had a lower ($p < 0.001$) water-holding capacity than the other treatments from the start of the trial until day 21, but by day 31, no differences ($p > 0.05$) were observed between treatments. The water-holding capacity of BS and PS declined steadily over the production cycles, but SH increased in water-holding capacity. The treatments converged towards similar water-holding capacity at the end of the production cycle.

At days 0 and 7, the mean bulk density varied widely ($p < 0.001$) between the litter treatments, increasing in bulk density from BS with the lowest bulk density, followed by PS and SH had the highest bulk density. Bulk density of BS was still the lowest at day 14 when compared to other treatments. From day 21 onwards, there were no differences in the bulk density between litter treatments. The SH had the smallest increase in bulk density and BS and PS increased at fairly similar rates.

Litter pH increased at similar linear rates for all three treatments as the production cycles progressed. No differences ($p > 0.05$) were observed in the mean pH across treatments at day 7 or from day 21 onwards. On day 14, the mean pH across treatments indicated that the lowest pH

Table 2: *Comparison of mean crude protein, acid detergent fibre, ether extract and ash (% dry matter basis ± SEM) at the commencement and conclusion of the production cycles*

	Bio-secure shavings	*Pine shavings*	*Sunflower hulls*
	Day 0		
Crude protein	$0.84^a \pm 0.06$	$0.88^a \pm 0.05$	$6.36^b \pm 0.03$
Ether extract	$0.64^a \pm 0.12$	$1.06^b \pm 0.23$	$8.75^c \pm 0.44$
Ash	$0.80^a \pm 0.41$	$0.48^a \pm 0.03$	$3.93^b \pm 0.83$
Acid detergent fibre	$83.92^c \pm 0.25$	$82.26^b \pm 0.51$	$63.84^a \pm 0.58$
	Day 31		
Crude protein	29.74 ± 1.04	28.78 ± 1.28	27.87 ± 1.25
Ether extract	1.70 ± 0.26	1.97 ± 0.33	1.95 ± 0.31
Ash	14.33 ± 0.56	13.31 ± 0.91	12.94 ± 0.63
Acid detergent fibre	31.57 ± 1.12	37.84 ± 2.01	34.87 ± 1.64

$^{a-c}$ Across treatments, means with no common superscripts differ significantly ($p < 0.05$) from each other. SEM = Standard error of the mean.

Table 3: *Litter moisture (%), water-holding capacity (%), bulk density (g L^{-1}), pH and caking (%) of the three litter types (±SEM) at various intervals across the production cycles*

Parameter	*Day*	*BS*	*PS*	*SH*	*ANOVA*
Litter moisture (%)	0	4.50 ± 0.25	4.40 ± 0.31	3.80 ± 0.14	NS
	7	5.00 ± 0.04	4.70 ± 0.09	4.90 ± 0.33	NS
	14	5.70 ± 0.21	5.90 ± 0.31	6.50 ± 0.24	NS
	21	6.40 ± 0.31	7.20 ± 0.54	7.40 ± 0.76	NS
	31	11.90^b ± 3.14	16.0^b ± 2.17	7.40^a ± 3.76	$p = 0.043$
Litter water-holding capacity (%)	0	3.13^b ± 0.11	3.28^b ± 0.11	1.67^a ± 0.03	$p < 0.001$
	7	2.58^b ± 0.06	2.65^b ± 0.07	1.68^a ± 0.01	$p < 0.001$
	14	2.13^b ± 0.07	2.12^b ± 0.05	1.74^a ± 0.11	$p < 0.001$
	21	2.19^b ± 0.09	2.04^b ± 0.05	1.81^a ± 0.02	$p < 0.001$
	31	1.90 ± 0.14	1.89 ± 0.25	1.90 ± 0.20	NS
Bulk density (g L^{-1})	0	50.17^a ± 2.10	122.30^b ± 7.37	170.30^c ± 3.90	$p < 0.001$
	7	93.50^a ± 4.42	143.20^b ± 6.00	195.80^c ± 2.84	$p < 0.001$
	14	181.33^a ± 20.24	225.80^b ± 5.50	235.00^b ± 9.39	$p = 0.003$
	21	301.17 ± 17.70	324.50 ± 11.94	326.50 ± 4.31	NS
	31	375.30 ± 6.97	403.30 ± 16.40	362.80 ± 9.79	NS
pH	7	6.11 ± 0.10	5.99 ± 0.06	6.07 ± 0.08	NS
	14	6.80^{ab} ± 0.16	7.14^b ± 0.25	6.55^a ± 0.17	$p = 0.037$
	21	8.04 ± 0.19	7.94 ± 0.33	7.70 ± 0.32	NS
	31	8.42 ± 0.24	8.47 ± 0.05	8.25 ± 0.22	NS

BS = Bio-secure shavings; PS = Pine shavings; SH = Sunflower hulls.
^{a,b,c} Across treatments, means with no common superscripts differ significantly ($p < 0.05$) from each other.
SEM = Standard error of the mean.

Table 4: *Mean percentage of litter caking (± SEM) between the treatments as compared at days 21 and 31.*

Litter type	*Day 21 (%)*	*Day 31 (%)*
Bio-secure shavings	60.01^a ± 8.17	87.60^b ± 4.17
Pine shavings	46.04^a ± 13.58	86.15^b ± 6.75
Sunflower hulls	57.92^a ± 13.46	81.88^b ± 6.37

^{a,b} Across treatments, means with no common superscripts differ significantly ($p < 0.05$) from each other. SEM = Standard error of the mean.

($p < 0.05$) was found for SH, the highest pH ($p < 0.05$) was found for PS and BS had a pH intermediate to these treatments ($p > 0.05$).

A very strong positive correlation coefficient for bulk density and litter caking was established ($r = 0.974$; $p < 0.001$). There was a weak correlation between litter caking and litter moisture ($r = 0.384$; $p = 0.044$). The litter caking among the treatments differed ($p < 0.001$) between days 21 and 31, but no differences ($p > 0.05$) were found between treatments on the same day of testing (Table 4).

The analysed nutrient content of the feed fed in each production cycle is depicted in Table 5. No differences ($p > 0.05$) were found within feed phases across production cycles for any of the parameters.

The broiler production parameters are depicted in Table 6. The use of SH as litter material resulted in higher ($p < 0.05$) mean slaughter weight (kg) and kilogram broilers produced per m^2, as well as average daily gain (g). Broilers on SH also presented with lower ($p < 0.05$) cumulative mortalities to seven days.

Litter content was observed during dissection of gizzards, but was not quantified. Acid detergent fibre in gizzard content and intestinal weight and length are depicted in Table 7. The mean ADF (on DM basis) in gizzard content across treatments differed between BS and SH ($p < 0.05$), while gizzard content from birds reared on PS had intermediate ADF content at 21 days. The mean empty proventriculus and gizzard weight of broilers across treatments on both sampling days indicated no differences ($p > 0.05$) between treatments. No differences ($p > 0.05$) were seen in the mean empty intestinal weight of broilers between treatments at 21 days. At 31 days, broilers reared on BS had proportionately heavier intestines ($p < 0.05$) when compared to those reared on SH, while broilers reared on PS had intestines of intermediate weight ($p > 0.05$). The mean duodenum, je-

Table 5: *Chemical composition (% 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

Table 6: *Summary of production performance at 33 days (± SEM) across the different treatments*

Production parameter	*Bio-secure shavings*	*Pine shavings*	*Sunflower hulls*	*ANOVA*
Kilogram m^{-2}	$37.62^a \pm 0.73$	$37.99^a \pm 0.49$	$40.40^b \pm 0.91$	$p = 0.010$
Total feed consumed (kg)	116924 ± 1528	116924 ± 3010	120961 ± 3854	NS
Mean slaughter weight (kg)	$1.75^a \pm 0.02$	$1.77^a \pm 0.02$	$1.85^b \pm 0.02$	$p = 0.036$
Feed conversion ratio	1.65 ± 0.03	1.65 ± 0.04	1.60 ± 0.03	NS
Average daily gain (g)	$52.42^a \pm 0.66$	$53.34^a \pm 0.64$	$55.44^b \pm 0.50$	$p = 0.004$
Production efficiency factor	301.80 ± 16.08	320.65 ± 20.60	330.47 ± 10.49	NS
Cumulative mortalities to 7 days (%)	$1.12^b \pm 0.14$	$1.08^b \pm 0.20$	$0.68^a \pm 0.07$	$p = 0.024$
Cumulative mortalities to 33 days (%)	5.35 ± 0.72	4.74 ± 0.89	3.97 ± 0.75	NS

^{a,b} Across treatments, means with no common superscripts differ significantly ($p < 0.05$) from each other.
SEM = Standard error of the mean.

Table 7: *Acid detergent fibre (ADF) in gizzard contents (% on DM basis), intestinal weight and length (± SEM) on different litter treatments at 21 and 31 days in the production cycle.*

	21 days				31 days			
Parameter	*BS*	*PS*	*SH*	*ANOVA*	*BS*	*PS*	*SH*	*ANOVA*
ADF in gizzard content	$14.52^a \pm 0.75$	$16.87^{ab} \pm 0.91$	$18.23^b \pm 0.69$	$p = 0.002$	12.96 ± 0.64	13.83 ± 0.75	14.91 ± 0.66	NS
Empty proventriculus and gizzard weight ($g\,kg^{-1}$ LW)	21.73 ± 0.27	22.57 ± 0.33	22.72 ± 0.43	NS	17.51 ± 0.31	16.84 ± 0.26	17.12 ± 0.30	NS
Empty intestinal weight ($g\,kg^{-1}$ LW)	29.16 ± 0.57	29.83 ± 0.58	31.88 ± 0.74	NS	$25.62^b \pm 0.46$	$23.95^{ab} \pm 0.48$	$23.46^a \pm 0.43$	$p = 0.005$
Duodenum and jejunum length ($cm\,kg^{-1}$ LW)	98.95 ± 1.36	104.59 ± 1.47	101.22 ± 1.74	NS	63.66 ± 0.86	63.64 ± 0.82	62.55 ± 0.90	NS
Ileum length (cm kg^{-1} LW)	73.96 ± 1.42	74.93 ± 1.41	74.78 ± 1.53	NS	49.01 ± 0.68	49.19 ± 0.57	47.48 ± 0.80	NS

BS = Bio-secure shavings; PS = Pine shavings; SH = Sunflower hulls. SEM = Standard error of the mean. LW = live weight.
^{a,b} Across treatments, means with no common superscripts differ significantly ($p < 0.05$) from each other.

Table 8: *Mean footpad dermatitis score out of five (± SEM) on different litter treatments at 21 and 31 days.*

Litter type	*Day 21*	*Day 31*
Bio-secure shavings	$2.121^a \pm 0.14$	$3.433^b \pm 0.15$
Pine shavings	$1.921^a \pm 0.29$	$3.075^b \pm 0.33$
Sunflower hulls	$1.996^a \pm 0.15$	$3.483^b \pm 0.24$

[a,b] Across treatments, means with no common superscripts differ significantly ($p < 0.05$) from each other. SEM = Standard error of the mean.

junum and ileum lengths on both days indicated no differences ($p > 0.05$) between treatments.

A strong correlation between litter caking and FPD ($r = 0.789$; $p < 0.001$) was established. As shown in Table 8, no significant differences were recorded for the occurrence of FPD between treatments on day 21 or 31. However, higher scores were recorded for FPD on day 31 compared to day 21 ($p < 0.001$).

4 Discussion

Several litter parameters, such as CP, ash, EE, ADF, bulk density, water-holding capacity and pH, converged to similar values towards the end of the production cycles, due to the homogenising effect of the addition of excreta, feed, feathers and water. The convergence of parameters have been reported in similar studies across a wide range of litter types (Davasgaium & Boodoo, 1997; Garcês *et al.*, 2013).

Litter moisture increased over the course of the production cycles and by the conclusion of the cycles, significant differences were observed between all three litter treatments. The moisture content of SH was the lowest, followed by BS, while PS had the highest moisture content. Decreased litter moisture has a direct financial advantage for the producer, because wetter litter requires higher ventilation speeds to dry and leads to increased costs (Dunlop *et al.*, 2015). Indirect benefits of decreased litter moisture include improved air quality and broiler welfare (Kheravii *et al.*, 2017b). Litter is classified as wet once it contains more than 25 % moisture (Collett, 2012), but litter moisture has varied widely (15-45 %) among studies evaluating different litter types (Groot Koerkamp, 1994; Hayes *et al.*, 2000; Miles *et al.*, 2011). Van der Hoeven-Hangoor *et al.* (2014) has shown that water activity is a more useful measure of water content present in litter than moisture content, which is less accurate. Water activity is the partial vapour pressure of a substance divided by the partial vapour pressure of pure water and thus a measure of the fraction of water not bound to solutes in the litter, as well as being closely related to the bacterial load in the litter. Financial constraints prevented the measure of water activity in the current study, as well as necessitating smaller sample sizes, which otherwise might have improved result reporting.

The water-holding capacity of BS and PS declined steadily over the course of the production cycle, whereas the water-holding capacity of SH improved. The water-holding capacity of SH remained significantly lower as compared to the other treatments, until day 21 of the study, after which no further differences were observed between treatments. The initial water-holding capacity values of the litter treatments in this study correspond to other studies (Garcês *et al.*, 2013; Jiménez-Moreno *et al.*, 2016). Dunlop *et al.* (2015) found that water-holding capacity of litter increased as the production cycle progressed, but the study utilised a different method of determining water-holding capacity. Litter in the current study was dried prior to testing water-holding capacity and a constant litter weight was used across the production cycle. Garcês *et al.* (2013) also found that water-holding capacity (on a DM basis, similar to this study) increased or decreased depending on the litter type. Dunlop *et al.* (2015) hypothesised that the true water-holding capacity of poultry litter would be a value in between that of compacted litter and litter allowed to settle under its own weight (expressed as L/m3 - the volume of water contained in one m3), due to chickens scratching and loosening some areas of litter, while other areas, such as around feeders, remain compacted.

The bulk density of the litter increased as the production cycle progressed. The initial bulk density of SH was comparable to SH in a study by Krizan *et al.* (2017), even though it was significantly higher than BS and PS bulk density. The changes between initial and final bulk density measurements provide comparable information to other sources. In the current study, litter bulk density of SH increased by 2.1 times, PS by 3.2 times and BS increased by 7.5 times. In a study by Garcês *et al.* (2013), litter bulk density increased on average 2.4 times. Bilgili *et al.* (2009) tested alternative litter sources to PS and found that the litter type with the highest initial bulk density had the lowest water-holding capacity and lowest moisture level, similar to SH in this study. Dunlop *et al.* (2016) concluded in a review that bulk density was not a crucial factor affecting litter susceptibility to wetness.

The litter pH increased at similar linear rates for all treatments as the production cycle progressed. At 14 days, PS had the highest pH and SH had the lowest pH. 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). As the litter pH shifted to alkaline, which occurred between days 14 and 21, other studies reported increased ammonia volatilisation, which negatively affected the air quality and increased pathogenic bacteria proliferation in litter (Kleyn, 2013; Wang *et al.*, 2016). Water activity measurements for bacterial monitoring as well as ammonia volatilisation would have been useful parameters to include in this study for air quality control during the latter half of the production cycle, but was prevented by financial constraints. The pH at the conclusion of the trial (8.2-8.5) was similar to results found in several other studies (Moore *et al.*, 1996; Terzich *et al.*, 2000; Garcês *et al.*, 2013).

When assessing litter caking, no significant differences were found between the treatments, but caking was significantly higher on day 31, as expected (Coufal *et al.*, 2006; Collet, 2012; Garcia *et al.*, 2012b). Litter containing larger particles (> 2.5 cm) tended to clump together more readily and propensity to caking was dependent on particle size as well as litter type (Grimes *et al.*, 2002; Kheravii *et al.*, 2017a). A strong positive correlation between caking of litter and bulk density, and a weak correlation between caking and litter moisture was observed in this study, supported by the findings of Dunlop *et al.* (2016). The incidence of FPD was increased by litter caking, which supports previous studies by Bilgili *et al.* (2009) and Garcia *et al.* (2012b). Some studies (Allain *et al.*, 2009; Youssef *et al.*, 2010; De Jong *et al.*, 2014) correlated FPD incidence with litter moisture, but these results were not repeated in the current study. Litter moisture, but not occurrence of FPD, differed significantly among treatments, concurring with the results of Škrbić *et al.* (2015).

Birds reared on SH had the highest slaughter weight during this trial, while no differences were found in total feed consumed, FCR, PEF or 33-day mortality between treatments. Most earlier studies found no differences among production parameters such as body weight, FCR (Brake *et al.*, 1992; Torok *et al.*, 2009), mortality (Toghyani *et al.*, 2010; Teixeira *et al.*, 2015) and total feed consumption (Swain & Sundaram, 2000) when different bedding materials were compared. Average daily gain of broilers reared on SH was significantly higher at 33 days, concurring with Jiménez-Moreno *et al.* (2016) that found an increase in average daily gain of 2.1 % when 5 % SH was included in broiler diets. Other litter types, such as PS, coir dust, rice husks and refused tea (Swain & Sundaram, 2000; Atapattu & Wickramasinghe, 2007; Cengiz *et al.*, 2011) have not shown similar performance effects. The 7-day mortalities of broilers were significantly lower when reared on SH. Since chicks from different aged flocks were evenly distributed and chick quality regarded as similar throughout the houses, SH may have had an effect on gut development of these broilers, as discussed later. The numerical higher PEF for SH in this study may be of economic importance, although an intensive cost analysis should be done to verify this. Production efficiency factor (PEF) is a robust criterion of evaluation in broiler production and an increase of 15 PEF points significantly improved profit margins (Samarakoon & Samarasinghe, 2012).

The much higher ADF content (on DM basis) in the gizzards (13–18.2 %) compared to the ADF content in the feed (5.6–7.8 %) indicated that the broilers consumed litter, concurring with Hetland *et al.* (2003). The initial ADF values of the three bedding materials differed significantly from each other, with SH having the lowest ADF concentration (63.84 % on DM basis) and BS the highest (83.92 % on DM basis). Furthermore, the gizzard ADF content of broilers reared on SH tended to be higher on day 21 (18.23 %) than those for BS (14.52 %), suggesting a possible higher intake of SH during early rearing. The initial EE concentration in SH was remarkably high at 8.75 % (on DM basis) compared to the 0.64 % and 1.06 % in BS and PS, respectively, and could have made a significant energy contribution when ingested. The higher initial CP (6.36 %) and ash (3.93 %) concentrations in SH compared to BS (0.84 % CP and 0.83 % ash) and PS (0.88 % CP and 0.48 % ash) could also have supported higher early growth of birds reared on SH. Inclusion of sunflower cake between 15 and 31 days of age improved weight gain and reduced Clostridium colony counts due to the positive effects of high fibre insoluble non starch polysaccharides on protein digestibility (Kalmendal *et al.*, 2011; Kheravii *et al.*, 2017b). It is thus possible that broilers reared on SH may have had better gut development early in the rearing period (Kimiaeitalab *et al.*, 2017) which led to the lower early mortality rates, but further research is needed to clarify this.

It is well-established that gizzard size is increased when coarse particles are added in the diet (Amerah *et al.*, 2008; Mateos *et al.*, 2012; Sacranie *et al.*, 2012; Wang-Li *et al.*, 2020). Due to these particles remaining in the gizzard for a longer period, gizzard contractions are increased, gizzard musculature hypertrophied and the pH of gizzard digesta is reduced (Hetland *et al.*, 2003; Kimiaeitalab *et al.*, 2017; Xu *et al.*, 2017). Broilers consuming hard, large particles of insoluble fibre such as oat hulls or had access to wood shavings were found to have heavier gizzards when compared to caged broilers without access to fibre (Sacranie *et al.*, 2012). Kimiaeitalab *et al.* (2017) found that feeding 3 % SH led to significant increases in broiler gizzard weight in the first three weeks of production, but these broilers were also caged without access to litter. In the current study, the empty proventriculus and gizzard weight of broilers did not differ across treatments, possibly due to the fact that all broilers had access to a source of fibre from litter. Viveros *et* *al*. (2009) did not find differences in digestive organ size when feeding broilers 4 % sunflower hulls in a diet consisting of 50 % more fibre than the control diet. It was unclear whether these broilers had access to litter in their brooding pens during the study. In studies comparing gizzard development across litter types, such as between PS and refused tea or comparisons between pelleted straw, shredded paper and PS, no differences were found (Atapattu & Wickramasinghe, 2007; Kheravii *et al*., 2017a).

The relative intestinal length (cm/kg body weight) of sacrificed broilers revealed no differences between treatments. Neither Kimiaeitalab *et al*. (2017) nor González-Alvarado *et al*. (2008) found an effect of fibre inclusion in the diet on absolute or relative intestinal tract length during the first 21 days of the production cycle. However, the relative intestinal weight of broilers reared on SH in the present study presented was significantly lower when compared to BS at 31 days-of-age. Due to relatively enlarged gizzards when exposed to fibre, intestinal weight may reduce as a gut adaptation to increased nutrient availability (Taylor & Jones, 2004). Decreased relative intestinal weight may also improve feed efficiency due to reduced intestinal maintenance costs (Xu *et al*., 2015). Amerah *et al*. (2008) found that intestinal weight at 21 days of production did not differ when varying sizes of coarse grain particles were fed to broilers. Discrepancy between broilers' responses to fibre is expected, as fibre sources differ in numerous properties, such as water-holding capacity, particle size, fibre percentage, degree of lignification, solubility and structure of polysaccharides in the fibre (Bach Knudsen, 2001; Svihus *et al*., 2002; González-Alvarado *et al*., 2008). Multiple studies have concurred that the inclusion of moderate amounts of fibre (2.5-5 %) improved gut development in broilers, as evidenced in the differences between empty intestinal weight in the current study (Hetland *et al*., 2003; González-Alvarado *et al*., 2007; Mateos, 2012; Jiménez-Moreno *et al*., 2016).

5 Conclusion

Broilers that have access to litter materials do consume their litter, which was evident in the increased ADF levels found in gizzard contents across treatments relative to the feed. The SH contained more nutrients based on proximate analysis, as compared to the other treatments. Broilers reared on SH showed reduced 7-day mortality, improved kilograms of broiler meat per m^2, average daily gain and slaughter weight at day 31. However, improvements seen with SH did not alter the commercially measured figures of PEF and FCR. Bio-secure pine shavings had no superior effect compared to PS, but SH might hold marginal benefits to farmers, if regionally available. Several parameters showed no differences between treatments and any benefit is more likely earlier in the production cycle, since 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.

Conflict of interest

The authors declare that they have no conflict of interest.

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