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Effect of Bedding Type on Bird Performance and Gut Health

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ABSTRACT

This study was conducted to compare wood shavings with Envirobed to determine the effect of bedding source on estimates of the amount of bedding consumed, and on some of the gut health indicators/microbial biomarkers (digesta dry matter content and the incidence of Campylobacter jejuni and Clostridium perfringens in the caecum of birds) and diet dry matter availability (assessed using titanium dioxide as a marker). A total of 144 Ross 308 one-day-old broiler chicks were randomly allocated to one of two bedding material treatments (Envirobed or wood shavings). Bird weight was greater for broilers kept on wood shavings, but birds consumed more Envirobed bedding and this was associated with drier digesta, suggesting a lower risk of Foot Pad Dermatitis with Envirobed. The caecal presence of either Campylobacter jejuni or Clostridium perfringens was not affected by the source of bedding. Rearing birds on wood shavings appears to be beneficial to broiler performance.

Keywords: Broilers, wood shavings, Envirobed, gut health.

INTRODUCTION

Pecking and litter scratching are natural behaviours of birds and suggest some ingestion of litter (VN Gupta et al., 2020). Indeed (Malone et al., 1983) reported that around 4% of poultry consumption consists of litter. In a separate experiment, using titanium dioxide as an inert marker, negative coefficients were calculated for fibre digestion and this was attributed to the consumption by birds of unknown amounts of wood-shaving bedding (Colleen et al., 2023). The consumption of bedding may in part be an attempt to meet a nutrient or structural fibre requirement of the bird (Hetland et al., 2005). The consumption of litter may have a beneficial effect on digesta quality and gut health, and different beddings may have different effects on these parameters. One alternative to wood shavings, although little used by poultry, is recycled paper waste marketed in the UK as Envirobed (Enviro Systems UK Ltd, Preston, UK). Like wood shavings, Envirobed is easy to handle and has good absorbent qualities (Hulet and Cravener, 2007). These were selected as indicators of possible increased moisture excretion, which would thus result in an increased risk of foot pad dermatitis. The two bacterial species investigated were selected as the former is a potential zoonosis and the latter a risk factor for necrotic enteritis and therefore poor welfare

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and economic performance (Estefania et al., 2024). Chickens continuously take up microorganisms from the surrounding environment during their growth cycle. The bedding material used in chicken houses is usually mixed with excreta and thus harbours a complex microbial community and is a potential influence on the microbiome of chicken gut (Yugal *et al.*, 2021). The prevalence of Campylobacter jejuni and Clostridium perfringens pathogens and the risk of disease can be lowered by a healthy gastrointestinal tract microbiota through colonization resistance and competitive exclusion (Zuami et al.,2020). The objective of this study was to compare wood shavings with Envirobed to determine the effect of bedding source on estimates of the amount of bedding consumed and on measures of gut health and the incidence of Campylobacter jejuni and Clostridium perfringens in the caecum of birds.

Material and methods

The study was subject to local review and conducted by the University of Reading's current animal research policy and confirmed to the United Kingdom's Animal (Scientific Procedures) Act 1986.

A total of 144 Ross 308 broiler chicks were collected on the day of hatching. The wings of the chicks were individually tagged with numbered identification tags and their weight was recorded. Tagged chicks were then randomly allocated to one of two bedding material treatments (Envirobed or Wood shavings). There were four replicate pens per treatment with 18 chicks in each pen. The four treatment pens were bedded with a layer of around 5 cm of either Envirobed or wood shavings. The size of each pen was 1X1.25 m and was lined with cardboard (approximately 60 cm height) to prevent draughts and reduce contact and transfer of feed and litter material between pens. A proprietary starter diet (Countrywide Chick Crumbs, Countrywide Farmers, Evesham, UK) was fed for 9 days before being abruptly changed to a grower diet from 10-21 days and a finisher diet was then fed until the completion of the experiment (at 38 d). The formulation of the grower and finisher diets is presented in Table 1 (according to the supplier's recommendation). All feed added and removed from pens was weighed and recorded and diet changes were conducted at the same time for all pens.

Birds and management

Lighting was via incandescent lights with 23 h continuous light per 24 h period for the first seven days, followed by 18 h continuous light (6 h darkness in each 24 h period). Feeders were maintained at a height equivalent to the birds' backs. Feed and water were supplied ad libitum (via hoppers and nipple drinkers, respectively). The target temperature for the whole room was 30°C for the first three days, dropping to 28°C on day 3, and then reducing by 1°C every three days until a temperature of 20°C was attained.

Retrieval of caecal contents

The caeca were removed using a sharp scalpel, flush with the ileo-caecal junction. The caecal contents were then squeezed into 2 ml Eppendorf tubes. These tubes were then 'snap frozen' in liquid nitrogen and then placed in dry ice before being transported to the laboratory where they were placed in a labelled bag and stored at -80°C pending molecular analysis for the presence of Clostridium perfringens and Campylobacter jejuni.

Culturing of caecal contents for microbial analysis

The sample of caecal contents for both Cl. perfringens and Campylobacter, serial di- lution (with sterile Brain Heart Infusion, BHI, broth) and plating of samples onto selec- tive media was done in an anaerobic cabinet. Plates were incubated (37°C) for 24 h in anaerobic conditions. The selective media (Oxoid, Basingstoke, UK) used were Cam- pylobacter Selective Agar for Campylobacter spp, and Perfringens Agar Base with egg yolk emulsion and Tryptose Sulphite Cycloserine (TSC) supplement for *Cl. perfringens*. Isolated colonies were counted on a colony counter.

DNA Extraction

Samples (0.15 g) of caecal contents were extracted with a Powersoil® DNA Isolation Kit (Qiagen,

Manchester, UK). The caecal sample was defrosted and processed accord- ing to the Powersoil® protocol, although approximately 0.15 g sample was weighed out rather than 0.25 g as suggested by the manufacturer as the caecal contents were a richer source of DNA than would be the case for soil. The resulting DNA was then transferred to an Eppendorf tube and its quality was determined to ensure that it was within the correct range to be able to undergo effective PCR. 1µl of C6 solution from the Powersoil® kit was used to calibrate the NanoDrop spectrophotometer (Thermo Fisher Scientific, Wilmington, Delaware, USA) used to detect quality. A sample of DNA (1 µl) was added to the NanoDrop and the concentration of DNA and the A260/280 nm were recorded. Any DNA concentration less than 5-10ng/µl with A260/280 <1.65 was discounted and not analysed by PCR.

Data relating to bird performance, digesta dry matter content and nutrient availability were subjected to analysis of variance (ANOVA) as repeated measures using a general linear model (GLM). Factors included in the model were treatment (d.f. =1), and time (d.f.=6) and the interaction between treatment and time. Results are presented as least square means with the standard error of the mean and associated P-value. All analyses were conducted using the MINITAB Vs. 17 software.

Effect of litter type on bird performance

The effect of bedding sources on bird performance is summarised in Table 2. Birds bedded on wood shavings rather than Envirobed tended to eat more (P=0.083), grew more quickly (P=0.040) and were likely therefore heavier (P<0.001). Their live weights increased at a faster rate when bedded on wood shavings rather than Envirobed (P<0.001). Although there was no effect of treatment on FCR, the increase in FCR with age tended to be greater when birds were bedded on wood shavings (P=0.088).

Effect of treatment on dry matter content g/kg in digestive tract

Digesta dry matter content was significantly higher in the crop and gizzard than in the ileum and colon (P<0.001, Figure 1). Digesta was drier in birds at 15 d of age compared with birds that were 36 d old (P=0.014). Although the effect of bedding source was not significant (P=0.707). At 15 d, there was no significant difference between bedding sources, but when the birds were 36 d old, birds kept on Envirobed had drier digesta than those kept on wood shavings.

Campylobacter jejuni

Campylobacter jejuni was not detected in any of the samples (Figure 2), and thus there was no evidence that the choice of bedding affected the establishment of this bacte- rium in the chickens' caeca.

Clostridium perfringens

The gene coding for the α toxin was present in all samples (Figure 3) with no evidence that choice of bedding had any effect on the presence in the chicken caecum of *Clos- tridium perfringens* type A.

Discussion

This experiment investigated the effects of two different bedding materials (wood shavings and Envirobed) on broiler performance, the presence of Campylobacter je- juni and Clostridium perfringens in the caecum of the chicken, and the dry matter con- tent of the digesta. Findings suggested wood shavings were beneficial for weight gain and bird live weight throughout the experiment (P=0.040; P<0.001) respectively. These results are in agreement with several studies which have reported that bedding type can influence bird performance (Jan and Peter, 2023, Jakub et al., 2023, Costa et al., 2021).

Absence of Campylobacter jejuni

There was no evidence of the presence (at a molecular level) of C. jejuni in any of the birds, which is good from a public and possibly bird health perspective. The chicks were from a commercial hatchery suggesting the hatchery is not the source of C. jejuni in birds entering the food chain. The paradigm of C. jejuni being often considered to be a harmless commensal bacterium of the chicken gut

(Walid et al., 2024), is beginning to be questioned for fast-growing broilers such as the Ross 308 used in this experiment. Faster-growing breeds have been observed to have a stronger immune inflammatory response to C. jejuni infection compared with slower-growing breeds, and this can lead to gut lining damage and subsequent diarrhoea (Humphrey et al., 2014). C. jejuni infection in fast-growing broilers may therefore, in addition to the public health risk, also increase the risk of reduced litter quality and increased incidence of hock burns and foot pad dermatitis (Humphrey et al., 2014)

This experiment was carried out in autumn/winter, perhaps accounting for the ab- sence of this experiment. According to (Yasiri et al., 2024) C. jejuni colonisation is greater in the summer months. (Jorgensen et al., 2011) Reported a significant difference in C. jejuni colonisation in different geographical locations of the UK and within regions there was seasonal variation with the highest prevalence in June and flocks reared in Northern Britain compared with central and South Britain $P<0.001$).

Presence of Clostridium perfringens type A

The Cpa gene coding for the α-toxin of Clostridium perfringens was observed in all the caecal contents, confirming the ubiquitous nature of this bacterial species. There was no evidence that the bedding on which the birds were kept (wood shavings or Envi- robed) affected this finding. These findings agree with the previous study in turkeys, and in studies by (Dahiya et al., 2006; and Gholamiandekhordi et al., 2006) who also tested the presence of the α-toxin in both flocks suffering from Necrotic Enteritis (NE) and a healthy flock without NE. More recent data indicate that the NetB toxin, rather than the α toxin, is the main virulent factor of Necrotic Enteritis (Keyburn et al., 2008; Keyburn et al., 2010). However, the presence of either the α-toxin or the NetB toxin (and certainly of the Cl. perfringens strains that produce the toxins) is not considered to be the sole cause of Necrotic Enteritis (Timbermont et al., 2011). Other predisposing factors (such as the presence of Eimeria infection and the feeding of wheat rather than maize) (Akhtar et al., 2012; Kim et al., 2017) need to be present to encourage the over-proliferation of the Cl. perfringens type A and the production of the α and NetB toxins.

In this experiment, focus has been on Cl. perfringens and C. jejuni. However, studies have suggested that it is the composition of the whole microbiome and perhaps dysbacteriosis (overgrowth of certain microorganisms) that is the major cause for dis- ease and consequent poor welfare (Muhammad Muneeb et al., 2024) rather than the presence or absence of particular bacterial species. The source and sink for these bacteria in the broiler is the litter, but there has been little if any investigation of the composition of the litter microbiome. The aim of the next study in this project was therefore to determine the effect of litter quality (fresh, or deliberately contaminated with poultry excreta) on the composition of the litter microbiome, and whether this was altered by the cereal source that the birds were fed.

Conclusion

This is the first report that has attempted to associate Envirobed and wood shavings consumption by poultry with measures of gut health. The caecal presence of either Campylobacter *jejuni* or Clostridium *perfringens* was not affected by the source of bed- ding used. Rearing birds on wood shavings did appear to be beneficial to broiler per- formance.

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Table 1: Formulation (g/kg as fed) of the grower and finisher diets.

Ingredient	Grower	Finisher
Corn	640	658.6
Soya Hi Pro	310	280
L-Lysine HCl	23	17
DL-methionine	29	26
L-threonine	13	11
Soya Oil	4	18
Limestone	12.5	12
Monocalcium phosphate	13.5	12.5
Salt	3	3
Sodium bicarbonate	1.5	1.5
Vitamin and Trace mineral premix	4	4
Titanium dioxide	5	**5**
Calculated nutrient composition (g/kg as fed)		
Crude Protein	209.2	195.6
ME (MJ/kg)	12.29	12.71
Calcium	9	8.6
Phosphorus	6.5	6.1
Lysine	13.1	11.7
Methionine	6.1	5.7
Total sulphur amino acids	9.5	8.8

Table 2: Effect of treatment on bird performance

	* Envirobed				** Wood shavings							
	Age of bird (d)				Age of bird (d)				p			
Parameters	**15**	**22**	**29**	**36**	**15**	**22**	**29**	**36**	**SEM**	**Treatment**	**Time**	**Interaction**
Feed Intake (g/bird/d)	36.0	89.4	116.8	178.9	35.7	90.0	125.8	192.2	4.42	0.083	<0.001	0.361
WeightGain (g/bird/d)	27.0	58.0	72.5	88.5	29.0	62.0	79.4	92.0	2.66	0.040	<0.001	0.818
†FCR (g feed/g gain)	1.33	1.54	1.61	2.02	1.24	1.45	1.58	2.08	0.0336	0.133	<0.001	0.088
Bird weight (g)	349	755	1277	1885	361	797	1349	2023	17.5	<0.001	<0.001	<0.001

*Envirobed: Birds were bedded on Envirobed throughout the experiment
**Wood shavings: Birds were bedded on wood shavings throughout the experiment
†FCR: Feed conversion ratio

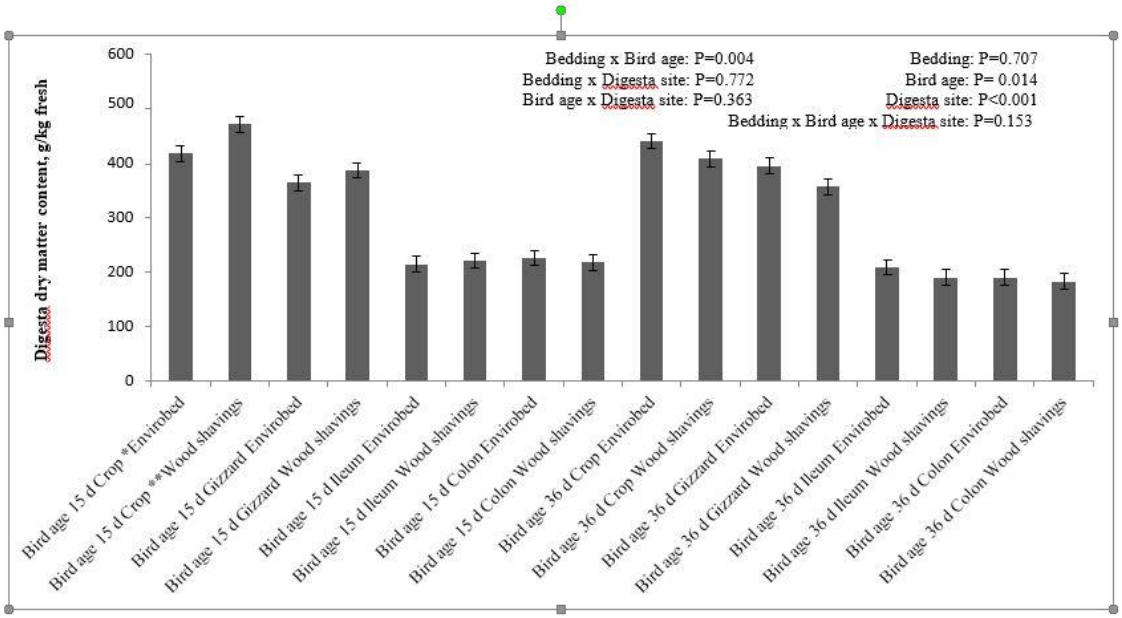

Figure 1: Digesta dry matter content

*Envirobed: Birds were bedded on Envirobed throughout the experiment

**Woodshavings: Birds were bedded on wood shavings throughout the experiment Figure 1: Effect of treatment on dry matter content g/kg in digestive tract

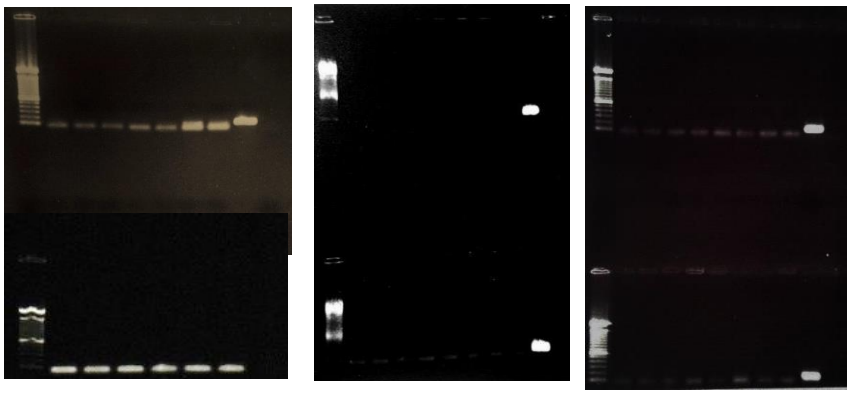

Figure 2: Left UV image of day 22 and right of day 38, both bedding types on both days indicated the absence of *ampylobacter jejuni*, measured against the 100bp ladder and the positive band, brightly lit on the far right of both images

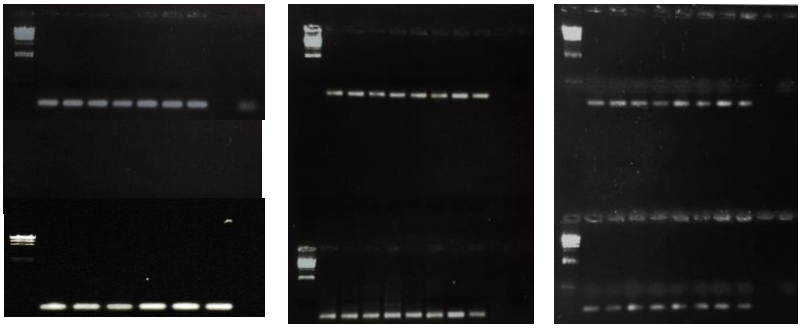

Figure 3: Left UV image on day 22 and right on day 38, indicated the presence of Type A alpha enterotoxin Clostridium perfringens in both bedding samples, measured against The lambda ladder (far left of each image)

تأثير نوع الفراش على أداء الطيور وصحة الأمعاء

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ملخص

أجريت هذه الدراسة لمقارنة تاثير فراش نشارة الخشب مع تأثير فراش البيئة الدارج على تقديرات كمية الفراش المستهلكة، وعلى بعض مؤشرات صحة الأمعاء/العلامات الحيوية الميكروبية (محتوى المادة الجافة في الجهاز الهضمي ومعدل الإصابة بـ Campylobacter jejuni وClostridium perfringens في الأعور لدى الطيور) وتوافر المادة الجافة في الغذاء (تم تقييمها باستخدام ثاني أكسيد التيتانيوم كعلامة). تم توزيع إجمالي 144 كتكوت دجاج لاحم عمره يوم واحد من Ross 308 بشكل عشوائي على إحدى معالجتي مادة الفراش.كان وزن الطيور أكبر بالنسبة للدجاج اللاحم الذي تم تربيته على نشارة الخشب، لكن الطيور استهلكت المزيد من فراش البيئة الدائم وكان هذا مرتبطًا بجفاف الجهاز الهضمي، مما يشير إلى انخفاض خطر التهاب باطن القدم مع Envirobed. لم يتأثر وجود Campylobacter jejuni أو Clostridium perfringens في الأعور بمصدر الفراش. يبدو أن تربية الطيور على نشارة الخشب مفيدة لأداء دجاج التسمين.

الكلمات الدالة: دجاج التسمين، نشارة الخشب، فراش البيئة الدائم، صحة الأمعاء.

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