Current Research in Poultry Science, 2012
ISSN 2152-2111 / DOI: 10.3923/crps.2012.

Influence of Housing System on Growth Performance and Intestinal Health of *Salmonella*-challenged Broiler Chickens

[1]F.B.O. Santos, [2]A.A. Santos Jr., [3]E.O. Oviedo-Rondon and [3]P.R. Ferket

[1]National Center for Swine and Poultry Research, Brazilian Agricultural Research Corporation-Embrapa, Concordia, SC, Brazil

[2]Faculty of Veterinary Medicine, Federal University of Uberlandia, Uberlandia, MG, Brazil

[3]Department of Poultry Science, North Carolina State University, Raleigh, NC, USA

Corresponding Author: F.B.O. Santos, National Center for Swine and Poultry Research, Brazilian Agricultural Research Corporation-Embrapa, Concordia, SC-Brazil, Br 153, Km 110 Concordia, SC 89700-000, Brasil Tel: +55 49 3441 0422

ABSTRACT

Rearing chickens on litter floors or in cages may influence their performance, especially when they are colonized by enteric pathogens, such as *Salmonella*. Male Ross 308 broilers were randomly assigned to 32 litter floor (litter) pens in a curtain-sided house or 32 cages (cages) in a total confinement house (25 birds/pen or cage). Birds were orally inoculated with 10^6 CFU of a cocktail of *S. enterica* subsp. *enterica* at three days of age. *Salmonella* populations (SP), body weights, feed conversion ratio and the weights of spleen and liver relative to body weight were determined at 14, 28 and 42 days of age. At each time point, characteristics of the intestinal segments were scored as an indicator of gut health on 32 birds per house. SP was higher in litter than cages treatment at 14 days of age which corresponded with a higher incidence of mucoid jejunum exudate. In contrast, cages had higher incidence of ileal grain chips than litter at 14 days, indicating inferior gizzard function. At 42 days of age, litter birds had higher breast meat yield, heavier body weight and improved cumulative feed conversion ratio than those in cages. Although, birds raised on litter floors showed greater 14 day *Salmonella* colonization than cage-reared birds, their digestion capacity appeared superior. Birds reared on litter floors had fewer undigested feed particles in their distal small intestine which correlates with enhanced growth performance and breast meat yield.

Key words: Broilers, intestinal health, housing, *Salmonella*

INTRODUCTION

Salmonella species can be easily found in the poultry farm environment and can as easily colonize the digestive tract of poultry (Seidavi *et al*., 2008). However, for humans these are pathogens well known for their role in a wide variety of diseases, ranging from enterocolitis to typhoid fever (Darwin and Miller, 1999; Mead *et al*., 1999). Furthermore, non-typhi strains are important zoonotic pathogens that cause foodborne disease in humans. Chickens, eggs and poultry products have been linked to outbreaks and cases human salmonellosis (Mead *et al*., 1999; Guard-Petter, 2001; Loongyai *et al*., 2001). Although, this pathogen can contaminate eggs through cracks in the shell (Williams *et al*., 1968), bacteria residing in the mucosal surfaces of the reproductive tract of the hen can access the egg during its formation and prior to oviposition

(Shivaprasad *et al.*, 1990). This mode of contamination is known as vertical transmission and can be especially difficult to control because the host is usually asymptomatic (Guard-Petter, 2001). Persistence of some serotypes, such as *S.* Enteritidis (SE) and *S.* Typhimurium (ST) in apparently healthy fowl leads to the introduction of contaminated raw products into the human food chain, thereby resulting in animal-human transmission (Williams *et al.*, 1968; Shivaprasad *et al.*, 1990; Guard-Petter, 2001). Consequently, it is crucial to control *Salmonella* at the farm level to reduce the risk of poultry product contamination (Blankenship *et al.*, 1993; Al-Nasser *et al.*, 2011).

Reece *et al.* (1971) has proposed rearing broilers in cages to prevent pathogenic contamination (i.e., *Salmonella*) arising from contact with their feces. In addition, Shini (2003) have shown that different housing systems (cage versus floor housing) affected immune response of broilers and Sekeroglu *et al.* (2009) showed that performance was significantly improved in broilers raised in conventional litter houses compared to free-range systems. Cage systems for broilers may also be a favorable alternative as the cost of housing spaces increases and the availability of pine shavings becomes scarce (Reece *et al.*, 1971; Andrews and Goodwin, 1973; Havenstein *et al.*, 1998). Nonetheless, the ingestion of structural particles such as wood shavings or oat hulls stimulates the development and function of the gizzard which helps to maintain normal intestinal motility and may improve feed efficiency (Hetland *et al.*, 2003; Santos *et al.*, 2008; Sundu *et al.*, 2008). Moreover, colonization of the hatchling intestine by commensal microorganisms is needed for the establishment of balanced intestinal microbiota (Lan *et al.*, 2005; Vicente *et al.*, 2007). Certainly, hatchlings inoculate themselves with microorganisms by ingesting particles within their reach, such as feed, water and litter (Lan *et al.*, 2005). Corrier *et al.* (1993) reported that chicks fed adult intestinal flora present in used litter are more resistant to *S. enteriditis* colonization.

Therefore, the purpose of the present study was to evaluate the effects of two types of housing, litter floors versus non-litter, on the *Salmonella* colonization, intestinal health, growth performance and meat yield of *Salmonella*-challenged broilers.

MATERIALS AND METHODS

Housing system: Two types of housing systems, a conventional experimental litter-floored house (litter) and a non-litter cage-based design (cages), the Broilermatic® cage system (Farmer AUTOMATIC of America, Inc. Register, GA), was used. The litter floor house had 32 pens (3.81×1.17 m pen^{-1}), each bedded with fresh pine wood shavings. This house was naturally ventilated by adjustable curtains on the sides. Two bucket tube feeders and one Plasson® drinker (Plasson UK Ltd, Billingshurst, UK) was installed per pen. In contrast, the non-litter house had 32 cages (1.93×1.19 m cage^{-1}) and was power ventilated. The cage flooring system was a soft nylon net, supported by metal bars. Each cage was equipped with two lateral galvanized feeders and four Plasson® nipple drinkers.

Diets: All diets were formulated using least-cost linear programming software to meet the dietary nutrient recommendations for Ross 308 broilers. The NRC recommendations for amino acids and energy were used as a reference for formulation (NRC, 1984). Experimental diets were formulated to be isocaloric and isonitrogenous. Three feed phases were used during the course of the experiment, starter (1-14 days), grower (15-28 days) and finisher (29-42 days). All feed was pellet-processed and fed in crumbles up to 42 days of age. The average particle size was 800 microns.

Bird husbandry: Eight hundred day-old (Ross 308) feather-sexed male chicks were randomly distributed among 32 pens or cages per house, such that 25 birds were housed per pen or cage. Then all birds were subjected to the same corn-soybean meal basal diet. The feeding program was divided into three phases depending on the age of the birds, resulting in: starter (from one to 14 days), grower (from 15 to 28 days) and finisher (29 to 42 days). At placement, all birds were individually identified with neck tags. At three days of age, chicks were individually challenged by oral inoculation with 1 mL containing 10^6 CFU of a mixture of *Salmonella enterica* subsp. *enterica* serotypes Typhimurium, Heidelberg, Newport and Kentucky. Feed and water were provided *ad libitum* throughout the entire study. All mortality was removed daily and the weights recorded so that an appropriate adjustment to feed conversion could be made.

Animal ethics: The birds were managed according to normal husbandry practices and euthanasia was performed with full consideration of animal welfare. The experiments were conducted according to the guidelines of the Institutional Animal Care and Use Committee (IACUC) of North Carolina State University.

Data collection: Average Body Weights (BW), Feed Consumption (FC), Feed Conversion Ratio (FCR) and mortality rates were determined at 14, 28 and 42 days of age. To determine BW all broilers from each pen or cage were individually weighed. Two birds per cage or pen were weighed and euthanized by cervical dislocation at 14, 28 and 42 days of age. A pooled sample of cecal contents from these two birds per pen or cage was used for *Salmonella* population analysis. Populations of *Salmonella* for each sample were determined using the Most Probable Number (MPN) technique as described by Santos *et al.* (2005) which is a method that enables an estimation of the population and differs from pre-enriched plate count method (Rathnayaka, 2011). The colonization estimates were expressed as $\log_{10}$ MPN per gram of cecal content. Additionally, spleen and liver weights were measured and evaluated from one bird per pen or cage at 14, 28 and 42 days of age. On the same days, the duodenum, jejunum and ileum were collected from 1 bird per pen or cage and the entire segments were scored double blind to the treatment to assess intestinal integrity according to parameters described in Table 1. This scoring system considered intestinal wall strength (friability), mucosal secretion consistency (exudate) and presence of undigested grain chips in the ileum.

Meat yield of four birds per pen or cage was determined at 42 days of age. Feed was withdrawn for twelve h before processing birds for carcass yield. Birds were processed in the North Carolina State University's experimental slaughter facility following departmental procedures. Carcasses were weighed without feet (empty carcass weight) and the yield was calculated as the ratio of empty carcass weight relative to 42 days BW after twelve h of feed withdrawal (the identity of individual broilers was maintained for 42-day live and carcass weights). In addition, processed drums, thighs, pectoralis minor, pectoralis major, wings and abdominal fat pad weights were

Table 1: Intestinal scoring system used to evaluate intestinal health of *Salmonella*-challenged broilers reared on litter floors or in cages[1], at 14, 28 and 42[2] days of age

Intestinal segment (e.g. duodenum, jejunum or ileum)			
Wall	Normal	Thin	Friable
Exudate	Normal	Mucoid	Watery
Digestion	Grains chips (Y/N)[3]		

[1]Broilermatic® cages system, Farmer automatic of America, Inc. Register, GA, [2]Birds were sampled before fasting period, [3] For ileum only

recorded on four birds per pen or cage. The skin was not removed before weighing the body parts. The yield for the body parts was expressed relatively to 42 days BW after twelve h of feed withdrawal.

Statistical analysis: All data, except the intestinal health scoring, were analyzed using the general linear models procedure for analysis of variance (ANOVA) of SAS® (SAS Institute Inc., 2005). The model used was:

$$Yi = \mu + Hi + i$$

where, H_i represented the house effect and ε_i the error term. Groups of birds within cages or pens served as the experimental units for statistical analysis. Variable means having a significant F-test ($p<0.05$) were compared using the least-square-means (lsmeans) function of SAS and were considered to be significant at $p<0.05$. Meat yield of the body parts collected was calculated as relative weight to the body weight of the sampled bird. Percentage meat yield and mortality data were transformed to arc sine of the square root to normalize the data distribution before statistical analysis. The intestinal health scores were analyzed using the PROC FREQ procedure of SAS® and variables were compared by the Chi-Square test of SAS® at $p<0.05$ (SAS Institute Inc., 2005).

RESULTS

***Salmonella* colonization:** At 14 days, there was a significant difference ($p<0.05$) in the *Salmonella* colonization levels between housing systems (Table 2). *Salmonella* MPN of cecal content was lower in the cage birds than those raised on litter (5.142 vs. 6.452 $\log_{10}$ MPN g^{-1} of cecal content, $p<0.05$). However, this effect was not significant at 28 and 42 days of age.

Table 2: *Salmonella* spp. colonization levels at 14, 28 and 42 days of challenged broilers reared on litter or in cages[1]

	Salmonella colonization, $\log_{10}$ MPN g^{-1} of cecal content		
House[2]	14 days	28 days	42 days
Litter	6.452[a]	4.268	3.298
Cages	5.153[b]	3.482	3.048
p-value	0.007	0.175	0.652
Pooled SEM (62)[3]	0.235	0.287	0.276

[1] Broilermatic® cages system. Farmer Automatic of America, Inc. Register, GA, [2]Values represent means of 32 birds per house (litter floor or cages), [3]Pooled SEM (62) = Standard error of the mean with 62 degrees of freedom, n = 64, Means within a column with different superscripts are significantly different ($p<0.05$)

Table 3: Relative weights of liver and spleen of *Salmonella*-challenged broilers reared on litter floors or in cages[1], at 14, 28 and 42 days of age

	14 days (g kg^{-1})[3]		28 days (g kg^{-1})		42 days (g kg^{-1})	
House[2]	Liver	Spleen	Liver	Spleen	Liver	Spleen
Litter	32.6	1.007	26.7[a]	1.082[a]	21.9	0.961
Cages	34.5	1.032	24.1[b]	0.952[b]	21.0	1.091
p-value	0.161	0.772	0.001	0.045	0.284	0.060
SEM (62)[4]	0.68	0.04	0.35	0.03	0.42	0.03

[1] Broilermatic® cages system. Farmer Automatic of America, Inc. Register, GA, [2]Values represent means of 32 birds per house (litter floors or cages), [3]Weights of liver and spleen were calculated relative to the body weight of the sampled bird, [4]Pooled SEM (62) = Standard error of the mean with 62 degrees of freedom, n = 64, Means within a column with different superscripts are significantly different ($p<0.05$)

Organ weights and intestinal health evaluation: There were no significant differences ($p>0.05$) in relative spleen or liver weights at 14 and 42 days of age (Table 3). However, at 28 days of age, liver (26.7 vs. 24.1 g kg^{-1} of BW, $p<0.05$) and spleen (1.082 vs. 0.952 g kg^{-1} of BW, $p<0.05$) relative weights were significantly higher in birds on litter compared to those in cages. Results from the intestinal health scores showed that no significant effect of housing system was found on intestinal segments' exudation (data not shown). However, wall strength findings showed a similar pattern for both duodenum and jejunum (Tables 4, 5). At 14 days of age the percentage of birds raised on litter that presented thinner walls for both intestinal segments was higher than the

Table 4: Duodenum wall characteristics of *Salmonella*-challenged broilers reared on litter floors or in cages[1], at 14, 28 and 42 days of age

	14 days (%)			28 days (%)			42 days (%)		
House[2]	Normal	Thin	Friable	Normal	Thin	Friable	Normal	Thin	Friable
Litter	46.9	3.1	0.0	43.8	6.3	0.0	48.4	1.6	0.0
Cages	48.4	1.6	0.0	40.6	9.4	0.0	42.2	7.8	0.0
Probability		0.001			0.05			0.01	

[1] Broilermatic® cages system, Farmer Automatic of America. Inc. Register. GA

Table 5: Jejunum wall characteristics of *Salmonella*-challenged broilers reared on litter floors or in cages[1], at 14, 28 and 42 days of age

	14 days (%)			28 days (%)			42 days (%)		
House[2]	Normal	Thin	Friable	Normal	Thin	Friable	Normal	Thin	Friable
Litter	37.5	12.5	0.0	46.9	3.1	0.0	37.4	7.9	4.7
Cages	42.2	6.3	1.6	34.4	15.6	0.0	34.4	7.8	7.8
Probability		0.001			0.001			0.01	

[1]Broilermatic® cages system, Farmer Automatic of America. Inc. Register. GA

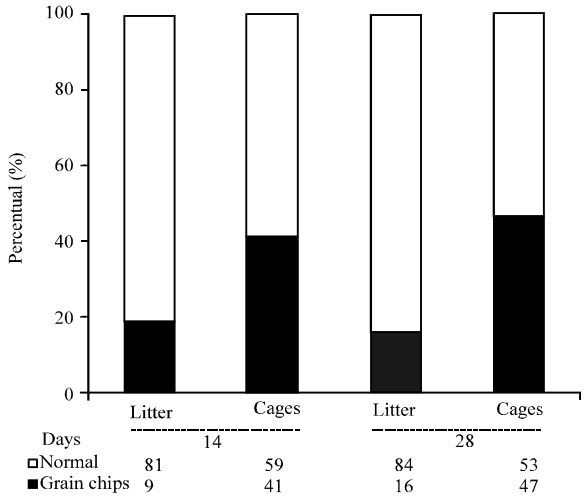

Fig. 1: Presence of grain chips in the ileum of *Salmonella*-challenged broilers reared on litter floors or in cages (Broilermatic® cages system. Farmer Automatic of America. Inc. Register. GA.) at 14 and 28 days of age (42 days data were missing and therefore, not included). Evaluations were performed on 32 birds per house (litter floors or cages). Pearson's Chi-square and table's probability for 14 days: 0.056 and 0.007 and 28 days: 0.036 and 0.006, respectively

Table 6: Body weight cumulative feed conversion ratio and mortality of *Salmonella*-challenged broilers reared on litter floors or in cages[1]. at 14. 28 and 42 days of age

	BW (g)				Feed conversion (g g^{-1})			Mortality (%)		
	----------days----------				----------days----------			----------days----------		
House[2]	1	1-14	1-28	1-42	1-14	1-28	1-42	1-14	1-28	1-42
Litter	41.0	437.0	1.549[a]	2.748[a]	1.308[b]	1.493[b]	1.836[b]	2.88	6.00[a]	12.440
Cages	41.2	444.0	1.511[b]	2.668[b]	1.449[a]	1.631[a]	1.935[a]	1.75	3.53[b]	13.030
p-value	0.569	0.113	<0.001	<0.001	<0.001	<0.001	<0.001	0.153	0.042	0.641
SEM (62)[3]	0.118	2.36	4.88	9.86	10.8	8.93	8.01	0.99	1.18	1.470

[1]Broilermatic® cages system. Farmer Automatic of America. Inc. Register. GA, [2]Values represent means of 32 pens (litter floors) or cages containing 25 chickens each, [3]Pooled SEM (62) = Standard error of the mean with 62 degrees of freedom, n = 64, Means within a column with different superscripts are significantly different ($p<0.05$)

Table 7: Carcass and body parts yield relative to body weight (BW) of 42 days-old *Salmonella*-challenged broilers reared on litter floors or in cages[1]

	Body parts weight relative to BW (%)			
House[2]	Carcass	Breast	Legs	Wings
Litter	80.73	24.91[a]	29.47[b]	11.11
Cages	80.76	24.02[b]	29.92[a]	11.31
p-value	0.897	0.001	0.004	0.147
Pooled SEM (254)[3]	0.001	0.001	0.001	0.001

[1]Broilermatic® cages system. Farmer Automatic of America. Inc. Register. GA, [2]Values represent means of 128 birds per house (litter floors or cages), [3]Pooled SEM (254) = Standard error of the mean with 254 degrees of freedom, n = 256, Means within a column with different superscripts are significantly different ($p<0.05$)

cage-reared birds (3.1 and 12.5 vs. 1.6 and 6.3%, respectively, $p<0.001$). But the effect was inverted at 28 and 42 days, so that the percentage of cage-reared birds showing friable jejunum walls was 3% higher than those reared on litter at 42 days of age (7.8 vs. 4.7%, $p<0.01$). Although, Fig. 1 shows that there was a higher incidence of grain chips observed in the ileum of caged birds than those on litter at 14 (40.6 vs. 18.8%, $p = 0.05$) and 28 days of age (46.9 vs. 15.6%, $p<0.01$), no significant effects of housing were found for ileum wall strength (data not shown).

Growth performance, livability and meat yield: The BW was significantly higher among birds reared on litter than in cages. By 28 days, birds in the litter group were heavier than those in the cages group (1549 vs. 1511 g, $p<0.05$) and this BW difference increased to 80 grams in favor of the litter group by 42 days (2,748 g vs. 2,668 g, $p<0.05$, Table 6). In addition, cumulative FCR of the litter birds was significantly ($p<0.05$) lower (i.e., improved) throughout the study than the cage birds (1.836 vs. 1.935 g:g, Table 6). At 28 days, cumulative mortality rates were higher among the litter than cage birds (6.0 vs. 3.53%, $p<0.05$, Table 6). Although, there was no significant house treatment effect on carcass yield (80.7 vs. 80.8%, $p>0.05$), the litter birds had higher breast meat yield (24.9 vs. 24.0%, $p<0.001$, Table 7) and lower leg meat yield (29.9 vs. 29.5, $p<0.05$, Table 7) than the cage birds.

DISCUSSION

The occurrence of foodborne salmonellosis poultry-related is an ongoing problem the poultry industry is facing. These infections are more a public health concern than an animal health

problem because, in most of the cases, poultry is asymptomatic, fact that greatly hinders the control of this pathogen at the farm level. *Salmonella* serotypes used in this study represent some of the most common serotypes isolated from cases of foodborne infections in the US (Centers for Disease Control, 2010). Table 3 shows that at 14 days cecal *Salmonella* colonization levels were significantly higher in birds reared on litter floors than those raised in cages, yet the effect was not significant at the end of the production cycle.

Broilers are known for their coprophagic behavior (Brownell *et al.*, 1969) and Nayak (2000) reported that bell-type drinkers can potentially disseminate *Salmonella* (10% of the drinker samples were positive for the pathogen), it was expected that the birds reared in the litter floor systems (direct contact with feces) would experience significant microbial recycling and higher colonization of enteric pathogens. Vicente *et al.* (2007) concluded in their work that acidified litter conditions reduced *Salmonella* horizontal transmission but was not able to eliminate *Salmonella* enteric colonization of broilers. Enteric pathogens, such as *S.* Typhimurium, are known to cause inflammation of the intestinal mucosa which is commonly associated with increased mucus secretion (Henderson *et al.*, 1999; Deplancke and Gaskins, 2001; Guarner and Malagelada, 2003). In addition, litter-reared birds had significantly higher liver and spleen weights and higher mortality rates at 28 days (Table 3, 6) compared to those reared in cages. Cell proliferation in lymphatic organs is accelerated as an immediate response to pathogenic invasion, resulting in enlarged organs (Turnbull and Snoeyenbos, 1974; Hassan and Curtiss, 1994; Henderson *et al.*, 1999). Although, in some cases innate immunity cannot prevent pathogen invasion and infection, frequently it is very effective to protect the chicks especially during their first week of life (Kogut, 2009). The protection from innate immunity may have reduced the need of a liver and spleen response at the first weeks of life but after this protective effect faded these organs increased their function. Analyzing the litter-raised broilers' response, it is likely that these birds were adapting to a pathogen invasion and fighting against it and even though the pathogen population decreased from 14 to 28 days of age, it was still possible to notice the results of infection.

Conversely, it has been demonstrated that exposure of *Salmonella*-challenged birds to a litter environment diverse in microbial communities can decrease shedding to the point that the pathogen was no longer detectable by market age (Brownell *et al.*, 1969; Santos, 2005; Santos *et al.*, 2008). In the present study, *Salmonella* colonization levels were detectable at market age but there were no statistically significant differences between the two housing systems. Santos *et al.* (2008) has reported that broilers challenged with *Salmonella* at three days of age and reared on litter floors have significantly lower populations of the pathogen by 42 days of age, when compared to broilers reared in cages. Furthermore, stable symbiotic microflora can be acquired by oral ingestion of materials from the bird's environment which exert a profound effect on intestinal health, immune response and performance of the host (Nurmi and Rantala, 1973; Guarner and Malagelada, 2003).

Throughout the study, the litter-reared broilers had heavier BW, lower FCR and, at 42 days, higher breast meat yield than the cage-reared birds (Table 6, 7). Similar results were found by Sogunle *et al.* (2008), where broilers raised on litter showed better feed conversion and lower mortality compared to those raised on litter. This difference in growth performance between the two housing types may be partly due to differences in drinker systems: Plasson® bell drinkers were used for the litter-reared broilers whereas nipple drinkers had to be used for the cage-reared broilers. Although, the type of drinker has been demonstrated to influence growth performance (Wabeck *et al.*, 1994), it is unlikely that this factor influences feed digestibility. Our results showed

that cage-reared broilers had a higher incidence of undigested grain chips in the ileum than those reared in litter floor pens at 14 and 28 days (Fig. 1). Clearly, grain digestibility in the cage-reared birds was reduced in comparison to those raised on litter floors.

Structural particles, such as pine shavings and feathers have been shown to enhance gizzard function and improve digestion of feed (Engberg *et al.*, 2004; Santos *et al.*, 2006). Studies in layers showed that hens voluntarily consume about four grams of wood shavings per day and their gizzards were three times heavier than cage-reared control birds (Hetland *et al.*, 2003). Moreover, the access to litter correlates with smaller particle size contents present in the small intestine compared to cage-reared control birds (Amerah *et al.*, 2009). In addition, ileal starch digestibility increases by 11% when broilers are fed a diet containing 6% wood shavings (Amerah *et al.*, 2009). Furthermore, Santos *et al.* (2008) reported that litter-reared birds not only have significantly heavier gizzards and proventriculi than cage-reared birds but they also have improved feed conversion efficiency and heavier breast muscle size relative to body weight. Therefore, non-digestible structural particles may influence nutrient absorption and improve feed efficiency in broilers.

In conclusion, enteric challenge during the first two weeks of life with *Salmonella* may be more pronounced in litter-reared broilers perhaps due to the immediate recycling of bacteria from droppings and particles in the environment. Furthermore, broilers reared in a conventional litter-based house had superior growth performance results when compared to those raised in a non-litter cage-based housing system. Lastly, litter floor housing may provide the bird with non-digestible structural particles that, upon ingestion, have remarkable effects on digestibility, feed efficiency, growth and meat yield. Further research is needed to determine how and which non-digestible structural components of the litter could benefit poultry.

ACKNOWLEDGMENTS

This work was supported by the USDA-IFAFS Grant and CAMAS, Inc. Le Center, MN. The authors thank Renee Plunske, Jamie Warner, Dr. Jean de Oliveira, Annette Israel, Michael Mann, Rizwana Ali and Robert Neely for their technical assistance during this study.

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