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Original Article

Different litter compositions influence broiler chicken locomotion

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Abstract

Background: Bedding material must absorb moisture, reduce impacts, and allow chicken to express their natural behavior reducing the occurrence of injuries in the footpad and joints, and improving carcass quality and performance. **Aims:** This study evaluated different bedding materials with different levels of inclusion of dried grass (*Zoysia japonica*) on the development of lesions in the locomotor system of broiler chickens. **Methods:** One thousand eight-day-old male chicks of the Cobb 500® were distributed in a completely randomized design with a 3 × 2 factorial scheme: three levels of grass inclusion and two bedding materials (100% wood shavings; 100% rice husks; 25% grass and 75% wood shavings; 25% grass and 75% rice husks; 50% grass and 50% wood shavings; 50% grass and 50% rice husks). The birds at 21, 28, 35, and 42 days of age were evaluated for pododermatitis. Ten birds from each repetition were identified and evaluated weekly from 21 to 42 days for Gait score, latency to lie and leg angle (valgus and varus). At 43 days of age, 60 birds of each treatment were slaughtered and evaluated for femoral degeneration and tibial dyschondroplasia. **Results:** Different litter compositions did not affect the incidence of lameness, tibial dyschondroplasia and spondylolisthesis. Pododermatitis scores increased after 28 days when including 50% of grass. **Conclusion:** Age contributes more to the development of locomotion injuries than does bedding material. It is recommended to use inclusions of dried grass only as bedding for young broilers.

Key words: Grass, Leg injuries, Litter, Poultry

Introduction

Broilers spend most of their life over litter; thus, it is essential to evaluate materials used in poultry farming. Bedding importance includes moisture absorption, thermal insulation, controlling the production of dust, moisture, and ammonia, preventing direct contact with the floor, softening impacts, incorporating excreta and feathers (Ekstrand *et al*., 1997; Yousef *et al*., 2010; Zikic *et al*., 2017). These factors increase animal comfort and allow them to behave naturally, such as scratching and dust bathing on bedding materials, which is essential for the bird's welfare.

The use of wood shavings as bedding is common in intensive broiler chicken production, especially in Brazil, however, this substrate often becomes difficult for producers to buy because of the high demand, leading to increased costs and reduced use that justifies the constant search and evaluation of alternative materials for poultry litter. Several possibilities have already been tested, including guinea grass, Eleusine grass, witchgrass, and Napier grass (Huang *et al*., 2009; Davis *et al*., 2010; Garcia *et al*., 2012). The use of grasses is of great relevance considering that previous studies have characterized them as an alternative material when well managed due to the low cost. In Brazil and other locations where the residues from the mowing of lawns are packaged in plastic bags and taken to landfills, there is a transformation of organic waste into an inorganic one, thus, the use of this material in poultry farming becomes a sustainable option. Bedding substrate and applied management are essential, for the health of birds, for reducing the occurrence of injuries to the foot-pad and joints and improving carcass quality and production parameters (Meluzzi *et al*., 2008; Bilgili *et al*., 2009; Zikic *et al*., 2017).

The growth rate of broiler chickens has increased with the intense genetic selection focused on performance, requiring only 5 weeks of life to reach 2 kg of weight (Tallentire *et al*., 2016). However, this compromises the development of the skeletal system and its integrity, causing increased bone porosity and impaired mineralization (Pratt and Cooper, 2018). Locomotor system lesions, particularly those that affect leg bones, cause chronic pain (Sorensen, 1989), and negatively affect the welfare of broilers (Almeida Paz *et al*., 2019). It also causes lameness, limiting birds' access to food resulting in considerable financial losses by increasing mortality and reducing productivity (Pines and Reshef, 2015).

Lameness can be caused by genetic, nutritional, environmental, or management factors. They involve the group size, accommodation system, quality of the space available, hygiene conditions, and bedding materials (Delezie *et al*., 2015). Thus, this study evaluated different bedding materials with different levels of inclusion of dried grass (*Zoysia japonica*) on the development of lesions in the locomotor system of broiler chickens and its impact on broilers welfare.

Materials and Methods

Experimental design

All procedures were approved by the Ethics Committee on Use of Animals (CEUA) of UFGD (protocol No. 07/2019). The experiment was carried out in the broiler experimental house of the Federal University of Grande Dourados, in the city of Dourados, Brazil, located in the south-central region of the state of Mato Grosso do Sul, Brazil, at 374 meters of altitude. The coordinates are 54°48'23.9" W and 23°13'18.52" S. The climate classification is humid mesothermal with hot and rainy summers and moderate and dry winters (Fietz *et al*., 2017). The broiler house was 50 m long, 10 m wide, and 3 m high, subdivided into 56 pens of 2.43 m^2 each (1.80 × 1.35 m) and was equipped with curtains, hoods, fans, evaporative plates (Plasson Livestock®), and a negative pressure temperature control with a panel to activate the equipment automatically. Each pen had a pendular drinker and a tubular feeder.

At the brooding phase (1 to 14 days old), the heating system comprised hoods with a 250 W infrared lamp for each pen. The light program adopted followed that of the Cobb broiler management manual (Cobb-Vantress, 2018).

At chicks first day-old, 1,080 Cobb 500® male chicks were housed in the poultry hause. They were first weighed using a digital scale (AD2000 Marte®) (average weight 48 ± 1.4 g) and distributed evenly for uniform weights across replicates. The experiment was completely randomized in a 3 × 2 factorial design: three levels of grass inclusion and two bedding materials. There were six replications each, totaling 36 pens with 30 birds each.

Six treatments were evaluated: 100% wood shavings, 100% rice husks, 25% grass and 75% wood shavings, 25% grass and 75% rice husks, 50% grass, and 50% wood shavings, and 50% grass and 50% rice husks. The inclusion of grass in the bedding materials was based on volume, measured in a 1 cubic meter box. The different bedding materials were replaced in the box by the volume corresponding to each grass inclusion.

The grass used as bedding was lawn grass (*Zoysia japonica*), cut to approximately 5 cm in length, and dried in the sun for three days in a haying process. A bedding height of 10 cm was turned over every two days during the brooding phase (1 to 14 days old), and daily during the growth and finishing phases (15 to 42 days old), using a rake in all treatments to avoid compaction. This practice aimed to simulate the reality of Brazilian farms, in which the turning is carried out as needed, in some cases daily.

The experimental diets were based on corn and soybean meal and supplied *ad libitum* and were formulated according to the production (brooding, growing, and finishing) phase, meeting the nutritional requirements proposed by Rostagno *et al*. (2017) (Table 1). The density of birds was 14 birds/m^2. The chicks were vaccinated in the hatchery against Marek, Bouba, Gumboro, and infectious bronchitis. Other management practices adopted during the conduction of the experiment were in accordance with those described in the Cobb strain manual (Cobb-Vantress, 2018), respecting the rules of animal welfare.

Data collection timeline

All the birds were weighed weekly and at 21, 28, 35, and 42 days of age, all bird legs were evaluated individually for pododermatitis. At 21 days of age, ten birds of each replicate were selected based on the average weight of each replicate, individually identified, and evaluated using the methodologies of gait score and latency to lie, as well as leg angulation (*valgus* and *varus*) (Weeks *et al*., 2002; Gonzales and Mendonça, 2006; Webster *et al*., 2008; Almeida Paz *et al*., 2019 (unpublished data)).

At 43 days of age, 360 birds were selected (60 of each treatment) based on the average weight of each replicate, identified with a leg band with no repeated and aleatory numbers making the evaluation blinded. Birds were subjected to eight-hour fasting and transported to the experimental slaughterhouse of the Federal University of Grande Dourados. The birds were euthanized by cervical dislocation and bled for at least 3 min. The carcasses were scalded in a water tank at 58°C for 2 min, followed by automatic plucking of carcasses. The evisceration and removal of feet and head were performed manually using specific knives for this procedure. The carcasses were cooled via immersion in water in two stages in a stainless-steel tank, with slow cooling lasting 18 min at 10-18°C (pre-chiller) and rapid cooling lasting 12 min at 0-2°C (chiller).

After cooling the carcasses, a macroscopic evaluation of femoral degeneration of both legs was performed as described by Almeida Paz *et al*. (2010). Tibial dyschondroplasia was evaluated as proposed by Almeida Paz *et al*. (2005).

Table 1: Composition and calculated nutritional values of the chicken diets for different production phases

Ingredients (%)	Days of age (phase)			
	1-7 days (brooding)	8-21 days (growing 1)	22-35 days (growing 2)	36-42 days (finishing)
Corn	55.08	59.26	61.88	66.58
Soybean meal 45%	38.31	34.79	31.58	27.37
Soybean oil	2.20	2.14	3.13	2.96
Limestone	0.92	0.91	0.82	0.77
Dicalcium phosphate	1.89	1.52	1.33	1.07
Common salt	0.35	0.35	0.35	0.35
DL-methionine	0.35	0.28	0.25	0.24
L-lysine HCl	0.28	0.26	0.19	0.23
Threonine	0.10	0.05	0.04	0.47
Choline chloride	0.07	0.06	0.06	0.04
* Vitamin supplement	0.10	0.10	0.10	0.10
** Mineral supplement	0.100	0.100	0.100	0.100
Calculated composition				
Metabolizable energy (kcal/kg)	2.950	3.000	3.100	3.150
Crude protein%	22.20	20.80	19.50	18.00
Digestible Met + Cis%	0.94	0.84	0.78	0.73
Digestible lysine%	1.31	1.17	1.07	1.01
Digestible threonine%	0.85	0.76	0.70	0.65
Calcium %	0.92	0.81	0.73	0.63
Available phosphorus%	0.39	0.34	0.31	0.27

* Composition vitamin supplement: Vitamin A 7,000 IU, vitamin D3 2,200 IU, vitamin E (minimum) 11 IU, vitamin K3 1.6 mg, vitamin B1 2.0 mg, vitamin B2 5.0 mg, vitamin B6 3.0 mg, vitamin B12 (minimum) 12 mcg, niacin 35 mg, pantothenic acid 13 mg, folic acid 800 mg/kg, and ** Mineral supplement composition: Copper 8 mg, iron 50 mg, iodine 1.2 mg, manganese 70 mg, selenium 0.2 mg, and zinc 50 mg. Met: Methionine, and Cis: Cysteine

Lameness evaluation

Gait score

A single trained evaluator performed the gait score test for the way birds walked in a linear one-meter path. Evaluations were performed at 21, 28, 35, and 42 days of age. The birds were taken to an empty pen, with only the test area marked, and the displacement of birds over a distance of 1 m was observed (Webster *et al*., 2008). The test started when the bird moved and ended soon as the bird stopped moving. According to the methodology described by Webster *et al*. (2008), a score scale of 0 to 2 was adopted, where score 0 was defined as: the bird walks normally without lameness and takes at least 10 uninterrupted steps; score 1: the bird walks with difficulty and takes between 6 and 9 steps; score 2: the bird has great difficulty in walking and takes less than 6 steps in 1 m.

Latency to lie

Two transparent containers (1 m length 35 cm width) were used, each with a shallow layer of water (3 cm), at room temperature (22 ± 3.7°C) (Almeida *et al*., 2017), where five birds from the same pen were placed in each container at a time. A digital timer (3308-021 Herweg®) was used to record the time required for each bird to make the first attempt to sit. When each bird attempted to sit, they were removed from the container. After 370 s, if a bird remained standing, the test was interrupted, and 370 s was assigned. If the chicken sat down right after being placed in the container, 0 s was assigned, according to the methodology of Weeks *et al*. (2002), and Almeida Paz *et al*. (2019).

Evaluation of locomotion injuries

Pododermatitis

A single trained evaluator performed foot injury assessments at 21, 28, 35, and 42 days of age by examining each foot pad, right and left, of all birds in the treatments. Foot injuries (redness and scabbing) were classified as follows: (0) no injury, (1) injury in less than 25% of the foot pad, (2) injury in 25 to 50% of the foot pad, and (3) injury in more than 50% of the foot pad (Martrenchar *et al*., 2002).

Valgus and varus

The lesions were characterized by unilateral, or bilateral (*valgus*), or medial (*varus*) deviations as described by Gonzales and Mendonça Jr. (2006). The deformity assessments *valgus* and *varus* consisted of assessing the angle of leg joints soon after the assessment of gait score. For such analysis, a single trained used evaluator a protractor and a ruler. The angle formed between the tibia and the third toe on the right and left legs was measured. A negative angulation characterizes the *varus* deformity; a positive angulation means *valgus* deformity. Deformities were classified as follows: score (0) up to 10°, score (1) from 11° to 20°, score (2) from 21° to 30°, and score (3) higher than 31°.

Tibial dyschondroplasia

After slaughter, the evaluation of tibial dyschondroplasia was performed macroscopically on both legs by a single trained evaluator. The growth cartilage thickening of these bones was evaluated, and scores were assigned according to the degree of injury, namely: score (0) no growth cartilage thickening, that is, a bird without injury; score (1) growth cartilage thickening varies between 1 and 3 mm, that is, there is an initial injury; and score (2) growth cartilage thickening greater than 3 mm, that is, a serious injury, according to the methodology described by Almeida Paz *et al*. (2005).

Femoral degeneration

To assess femoral degeneration, after slaughtering the birds, continuous numbered identified carcass had their legs removed, and the heads of the right and left femurs were examined macroscopically by a single trained evaluator and scores ranging from 0 to 2 were assigned. Score (0) means femur head without injury, score (1) means femur head with an initial injury, and score (2) means femur head with severe injury, according to the method described by Almeida Paz *et al*. (2010).

Spondylolisthesis

After slaughter, the carcasses were carefully identified, packaged, and stored at -15°C for 90 days. Then, spondylolisthesis was evaluated by a single trained evaluator analyzing the vertebrae integrity. For this, the spine was sawn lengthwise in the median region using a band saw. Each bird was classified to deformity and non-deformity groups as described by Paixão *et al*. (2007).

Bone analysis

Seedor index, resistance to breakage, and bone ashes (tibia)

After slaughtering the birds, the left thighs were identified, packaged, and stored at -15°C for 120 days. To determine the percentage of dry matter, the tibiae were carefully boned, dried at room temperature for 24 h, and weighed using a digital scale (AD2000 Marte® capacity 2010 g resolution 0.01 g) before and after being placed in an oven at 105°C for 24 h.

Before placing the tibiae in the muffle oven, tibia length was measured using digital calipers (Mitutoyo®, Japan) to obtain the Seedor index by dividing the bone weight by its length, as proposed by Seedor (1995).

The samples were subjected to incineration in the muffle oven at 600°C for 8 h to determine ash content in lipid extraction, as described by Yan *et al*. (2005).

The right tibiae were subjected to bone strength assessment through shear force analysis using a TA Texture analyzer with an XT Plus and Blade Set HDP/BS probe regulated to a speed of 4 mm/s in the pre-test and at a distance of 10 mm. The equipment was adapted to allow the free span of bone diaphysis by 6.0 cm, preventing the interference of length on the results.

Statistical analysis

The data obtained by analysis of lameness and locomotion injuries were score data therefore, these data do not have a normal distribution. Thus, the generalized linear model theory proposed by Nelder and Wenderburn (1972) was used and the SAS GLIMMIX (SAS, 2003) procedure was applied. The data distribution was GAMMA, except for spondylolisthesis data, in which the BINARY distribution was used. The GAMMA distribution assumes that data residues have exponential behavior, while the BINARY distribution assumes that residues have a Poisson behavior.

For latency to lie, gait score, and *valgus* and *varus* the statistical models included bedding material, level of grass inclusion, week of age, and their interactions as fixed factors. Animal, nested within each treatment, was included as a random factor in all models (command RANDOM).

For pododermatitis, tibial dyschondroplasia, femoral degeneration, and spondylolisthesis the statistical models included bedding materials, level of grass inclusion, and their 2-way interactions as fixed factors.

To obtain the coefficients and intercepts of regressions, the SOLUTION command of the GLIMMIX procedure was used. To compare the averages by "*lsmeans*" test, the "*estimates*" obtained were used in "inverse link" functions and adjustments (pdiff ilink lines) of the GLIMMIX procedure. Data were expressed as estimates of score data that corresponded to values estimated by the GLIMMIX statistical package. They are numerical values, that is, the higher the number, the greater the injury, as also described by Kiani *et al*. (2019).

For Seedor index, resistance to breakage, and bone ashes the data were checked for the normality of the residuals using the Shapiro-Wilk test and homogeneity of the variances using the Levene test. Subsequently, they were subjected to analysis of variance through the MIXED procedure of the SAS (SAS 9.3). The significance used for all analyses was 5% probability.

Results

Latency to lie and gait score

In welfare assessments using latency to lie and Gait score, there was no interaction between bedding material and grass inclusion levels. Regarding the isolated effects of bedding and grass inclusion, there was no effect on the evaluated locomotion injuries. There was a significant age effect (Table 2). In the evaluation using latency to lie, the variable "age" showed a decreasing linear effect of age. Thus, the older the birds, the lesser they could stand in the water container. The gait score showed a positive linear correlation between birds' age and the scores (Table 2).

Pododermatitis

A significant interaction was observed between bedding types and grass inclusion regarding the evaluation of pododermatitis in all ages evaluated, except for the evaluation at 35 days (Table 3).

At 21 days of age, the bedding made up of 50% grass and 50% wood shavings resulted in lower pododermatitis scores on both legs. For rice rusks there was no difference regarding the different levels of grass inclusion on both legs. Comparing the substrates in the different levels of grass inclusion, rice husks induced lower scores of pododermatitis in both legs (Table 3).

At 28 days of age, the bedding without grass inclusion in wood shavings promoted lower pododermatitis scores in the left leg when compared to inclusion of 25% and 50%. In the case of grass inclusions in rice husks, this result did not repeat.

Table 2: Latency to lie and gait score of broilers raised on two types of litter with different levels of grass inclusion and age effects in variables

	Latency to lie (s)			Gait score[1]		
Grass (%)	0	25	50	0	25	50
Wood shaving	244.34 (8.56)	249.95 (8.99)	250.13 (7.60)	0.525 (0.04)	0.495 (0.04)	0.474 (0.04)
Rice husk	261.34 (8.28)	240.71 (8.54)	263.02 (8.40)	0.504 (0.04)	0.534 (0.04)	0.512 (0.04)
	P-values[3]					
Bedding		0.31			0.61	
Grass		0.40			0.84	
Age		<0.0001			<0.0001	
Bed*Grass		0.26			0.74	
Bed*Age		0.53			0.90	
Grass*Age		0.10			0.87	
Bed*Grass*Age		0.35			0.60	
	Regression					
Age equation		y=-3308.10x+366.46			y=0.0258x-0.5707	
MSE[2]		a(340.43) b(34.61)			a(0.010) b(0.21)	
P-value[3]		<0.0001			0.02	

[1] The values presented in the table correspond to estimates obtained from the GLIMMIX statistical package of SAS 9.3 and have a numerical character (the highest correspond to the worst injury) followed by the corresponding mean standard error in parentheses, [2] Mean standard error, and [3] P-values considered significant at 5%

Table 3: Pododermatitis of broilers raised on litter with different levels of grass at different ages

	Left leg				Right leg			
21 days[1]								
Grass (%)	0	25	50	Mean	0	25	50	Mean
Wood shaving	0.39 (0.03) Aa	0.37 (0.03) Aa	0.21 (0.03) Ba	0.140 (0.01)	0.43 (0.030) Aa	0.333 (0.033) Aa	0.211 (0.027) Ba	0.099 (0.017)
Rice husk	0.13 (0.03) Ab	0.17 (0.03) Ab	0.12 (0.03) Aa	0.325 (0.01)	0.113 (0.030) Ab	0.125 (0.029) Ab	0.058 (0.029) Ab	0.325 (0.017)
Mean	0.26 (0.02)	0.27 (0.02)	0.16 (0.02)		0.272 (0.021)	0.229 (0.022)	0.134 (0.020)	
	P-values[2]							
Bed		<0.0001				<0.0001		
Grass		0.01				<0.0001		
Bed*Grass		0.03				0.02		
28 days[1]								
	Left leg				Right leg			
Grass (%)	0	25	50	Mean	0	25	50	Mean
Wood shaving	0.31 (0.03) Aa	0.17 (0.03) Ba	0.19 (0.02) Ba	0.08 (0.01)	0.30 (0.03)	0.22 (0.03)	0.18 (0.02)	0.23 (0.01) a
Rice husk	0.08 (0.03) Ab	0.07 (0.02) Aa	0.07 (0.02) Ab	0.22 (0.01)	0.09 (0.03)	0.07 (0.03)	0.05 (0.02)	0.07 (0.01) b
Mean	0.19 (0.02)	0.12 (0.02)	0.13 (0.02)		0.19 (0.02) A	0.15 (0.02) A	0.12 (0.02) B	
	P-values[2]							
Bed		<0.0001				<0.0001		
Grass		0.01				0.02		
Bed*Grass		0.03				0.30		
35 days[1]								
	Left leg				Right leg			
Grass (%)	0	25	50	Mean	0	25	50	Mean
Wood shaving	1.13 (0.05)	1.25 (0.05)	1.22 (0.05)	1.20 (0.03) a	1.06 (0.05)	1.19 (0.05)	1.15 (0.04)	1.13 (0.03) a
Rice husk	0.58 (0.05)	0.85 (0.05)	0.85 (0.05)	0.76 (0.03) b	0.56 (0.05)	0.82 (0.05)	0.83 (0.05)	0.73 (0.03) b
Mean	0.85 (0.04) B	1.05 (0.04) A	1.04 (0.03) A		0.81 (0.03) B	1.00 (0.03) A	0.99 (0.03) A	
	P-values[2]							
Bed		<0.0001				<0.0001		
Grass		0.01				0.01		
Bed*Grass		0.17				0.22		
42 days[1]								
	Left leg				Right leg			
Grass (%)	0	25	50	Mean	0	25	50	Mean
Wood shaving	1.65 (0.05) Ca	2.29 (0.06) Aa	2.00 (0.05) Ba	1.98 (0.03)	1.53 (0.06) Ca	2.33 (0.06) Aa	1.91 (0.05) Ba	1.93 (0.03)
Rice husk	1.15 (0.05) Bb	1.75 (0.05) Ab	1.76 (0.05) Ab	1.56 (0.03)	1.04 (0.06) Bb	1.57 (0.05) Ab	1.66 (0.05) Ab	1.42 (0.03)
Mean	1.40 (0.04)	2.02 (0.04)	1.88 (0.04)		1.29 (0.04)	1.95 (0.04)	1.79 (0.040)	
	P-values[2]							
Bed		<0.0001				<0.0001		
Grass		<0.0001				<0.0001		
Bed*Grass		0.02				0.01		

[1] The values presented in the table correspond to estimates obtained with the GLIMMIX statistical package of SAS 9.3 and have a numerical character (the highest corresponds to the worst injury) followed by the corresponding mean standard error in parentheses. Values followed by capital letters on rows differ statistically. Values followed by small letters in columns differ, and [2] P-values considered significantly at 5%

Comparing the substrates in the different levels of grass inclusion rice husks induced lower scores of pododermatitis in both legs for 0% and 50% (Table 3).

On the right legs at 28 days of age, there was no significant interaction between bedding type and grass inclusion. However, there was an isolated effect of bedding material: the rice husk had lower scores than shaving ones, as well as an isolated grass effect, where the lowest scores occurred by including 50% of this grass.

At the 35-day evaluation, there was no interaction between bedding and grass inclusions. However, there was an isolated effect of bedding material and grass inclusion. When included 25% or 50% of grass in both bedding materials, there are higher scores of pododermatitis in both legs. By contrasting wood shavings and rice husks regardless of grass inclusion, there were lower pododermatitis scores in both legs of birds on rice husks.

At 42 days, there was an interaction between grass inclusion and bedding substrate in both legs. For both legs, including 25 or 50% grass in wood shavings and rice husks showed higher severity of pododermatitis than the pure bedding substrates. When substrates were compared to each other, the birds housed on rice husks had fewer pododermatitis lesions on both legs for each grass percentage.

Valgus and *varus*

There was no significant interaction between bedding

Table 4: Evaluation of *valgus* and *varus* in broilers raised on litter with different levels of grass inclusion

	Valgus and *varus* left[1]			*Valgus* and *varus* right[1]		
Grass	0	25	50	0	25	50
Wood shaving	1.06 (0.05)	1.08 (0.06)	1.05 (0.05)	1.13 (0.05)	1.15 (0.06)	1.07 (0.52)
Rice husk	1.1 (0.05)	1.18 (0.05)	1.05 (0.056)	0.97 (0.05)	1.27 (0.05)	1.09 (0.58)
	P-values[2]					
Bed		0.34			0.87	
Grass		0.37			0.01	
Age		<0.0001			0.01	
Bed*Grass		0.71			0.06	
Bed*Age		<0.0001			0.16	
Grass*Age		0.64			0.43	
Bed*Grass*Age		0.62			0.06	
	Bed*Age equations			Age equation		
	Husks y=0.0514x-0.508			y=0.0911x-1.210		
P-value equation[2]	P<0.0001			P<0.0001		
MSE[3]	a (0.01) b (0.12)			a (0.32) b (0.01)		
	Shavings y=0.0256x+0.1032					
P-value equation[2]	P=<0.0001					
MSE[3]	a (1.07) b (0.07)					
	Age effect (rice rusk)[1]			Grass effect[1]		
21	28	35	42	0	25	50
0.48 D	1.07 C	1.269 B	1.61 A	1.05 B	1.21 A	1.08 B
	Age effect (shavings)[1]					
21	28	35	42			
0.76 C	0.82 C	1.23 B	1.44 A			

[1] The values correspond to estimates obtained by the GLIMMIX statistical package of SAS 9.3 and have a numerical character (the highest corresponds to the worst injury) followed by the corresponding mean standard error in parentheses. Values followed by capital letters in rows differ statistically, [2] P-values considered significant at 5%, and [3] a Mean standard error for angular coefficient b Mean standard error for intercept

Table 5: Tibial dyschondroplasia and femoral degeneration in broilers reared on litter with different grass inclusion levels

	Tibial dyschondroplasia[1]								Femoral degeneration[1]							
	Left leg				Right leg				Left leg				Right leg			
Grass	0	25	50	Mean	0	25	50	Mean	0	25	50	Mean	0	25	50	Mean
Wood	0.59	0.66	0.44	0.56	0.61	0.58	0.59	0.59	1.38	1.58	1.52	1.49	1.47	1.50	1.37	1.45
shaving	(0.12)	(0.13)	(0.11)	(0.07)	(0.13)	(0.14)	(0.12)	(0.07)	(0.11) Aa	(0.12) Aa	(0.10) Aa	(0.06)	(0.11)	(0.12)	(0.10)	(1.18)
Rice husk	0.49	0.41	0.34	0.41	0.40	0.45	0.43	0.428	1.40	1.38	1.90	1.55	1.41	1.43	1.56	1.47
	(0.13)	(0.13)	(0.12)	(0.07)	(0.13)	(0.13)	(0.13)	(0.07)	(0.12) Ba	(0.12) Ba	(0.11) Aa	(0.06)	(0.12)	(0.12)	(0.11)	(1.20)
Mean	0.54	0.53	0.39		0.50	0.51	0.51		1.39	1.48	1.70		1.44	1.46	1.47	
	(0.09)	(0.09)	(0.08)		(0.09)	(0.10)	(0.091)		(0.08)	(0.08)	(0.08)		(1.46)	(1.52)	(0.07)	
	P-values[2]								P-values[2]							
Bed		0.1564				0.1335				0.519				0.4167		
Grass		0.4205				0.996				0.0193				0.4828		
Bed*Grass		0.8321				0.954				0.0474				0.5307		

[1] The values correspond to estimates obtained from the GLIMMIX statistical package of SAS 9.3 and have a numerical character (the highest corresponds to the worst injury) followed by the corresponding mean standard error in parentheses. Values followed by capital letters in rows differ statistically. Values followed by small letters in columns differ statistically, and [2] P-values are considered significant at 5%

types and grass inclusion in the assessment of angular deformities.

For *valgus* and *varus* in the left leg, there was a significant interaction between bedding types and age, i.e., an increasing linear effect for both materials tested (Table 4). Thus, the scores increased over the weeks of the bird's life.

In age and bedding interaction, all weekly evaluations differ from each other in treatments with rice husks. This represents increasing values of scores during evaluations. It evidences higher scores of *valgus* and *varus* locomotor problems at 42 days.

In shavings, the first two assessments were not different (21 and 28 days). There was an increase in the last two assessments (35 and 42 days), also demonstrating higher angular defect scores at 42 days.

For *valgus* and *varus* in the right leg, there was no interaction between bedding types and grass inclusion. However, there were isolated grass and age effects. Age had an increasing linear effect. For grass inclusion, the estimated scores showed that the inclusion of 25% grass in the substrates represented higher *valgus* and *varus* defect scores when compared to the other levels of inclusion.

Tibial dyschondroplasia

For tibial dyschondroplasia, there was no significant interaction between bedding material and grass inclusion levels in both legs, nor isolated effects of grass inclusion and bedding material (Table 5).

Femoral degeneration

For femoral degeneration, there was a significant interaction between bedding types and grass inclusion. There were higher scores of femoral degenerations in the left leg of treatments with the inclusion of 50% of grass in rice husks. In the right leg, however, there was no significant interaction or isolated effects of grass inclusion and bedding (Table 5).

Spondylolisthesis

There was no significant interaction between bedding material and grass inclusion levels, nor isolated effects of grass inclusion and bedding material on the incidence of spondylolisthesis (Table 6).

Table 6: Evaluation of spondylolisthesis in broilers raised on litter with different levels of grass inclusion

	Spondylolisthesis[1]			
Grass	0	25	50	Mean
Wood shaving	0.64 (0.37)	1.38 (0.42)	0.49 (0.30)	0.84 (0.21)
Rice husk	1.27 (0.42)	0.58 (0.39)	0.77 (0.34)	0.87 (0.22)
Mean	0.95 (0.28)	0.98 (0.28)	0.63 (0.23)	
	P-values[2]			
Bed	0.91			
Grass	0.54			
Bed*Grass	0.18			

[1] The values correspond to estimates obtained with the GLIMMIX statistical package of SAS 9.3 and are followed by the corresponding mean standard error in parentheses, and [2] P-values considered significant at 5%

Seedor index, resistance to breakage, and bone ashes (tibia)

There was no significant interaction between bedding material and grass inclusion levels, nor isolated effects of grass inclusion and bedding material on the percentage of dry matter, mineral matter (ash), seeder index, and resistance to breakage in the tibias of broilers (Table 7).

Table 7: Dry matter, mineral matter (bone ash), *Seedor* index, and resistance to breakage in tibias of broilers raised on litter with different levels of grass inclusion

Variable	Substrate	Grass			Mean	MSE[7]	P-values[8]		
		0	25	50			Bed	Grass	Bed*Grass
DM[1] (%)	Rice husk	97.45	97.53	97.38	97.46	0.07	0.17	0.95	0.55
	Wood shaving	97.21	97.17	97.39	97.26				
	Mean	97.33	97.35	97.38	97.36				
MM[2] (%)	Rice husk	41.95	42.65	42.43	42.34	0.03	0.07	0.32	0.18
	Wood shaving	42.93	41.23	42.35	42.17				
	Mean	42.44	41.94	42.39	42.25				
SI[3]	Rice husk	1.00	0.97	1.01	1.00	0.01	0.69	0.75	0.39
	Wood shaving	1.03	1.01	0.98	1.01				
	Mean	1.01	0.99	1.00	1.00				
R[4] (Kgf)	Rice husk	12.79	13.01	11.94	12.58	0.39	0.11	0.48	0.99
	Wood shaving	11.57	11.74	10.66	11.33				
	Mean	12.18	12.38	11.30	11.93				
D[5] (mm)	Rice husk	3.50	2.77	2.65	2.97	0.15	0.26	0.32	0.35
	Wood shaving	3.42	2.94	3.63	3.33				
	Mean	3.46	2.86	3.14	3.18				
T[6] (mm)	Rice husk	1.75	1.50	1.59	1.61	0.08	0.42	0.59	0.94
	Wood shaving	1.83	1.63	1.81	1.76				
	Mean	1.79	1.56	1.70	1.69				

[1] DM: Dry matter, [2] MM: Mineral matter, [3] SI: *Seedor* index, [4] Resistance, [5] Diameter, [6] Thickness, [7] MSE: Mean standard error, and [8] P-values considered significantly at 5%

Discussion

In the present study it was possible to verify a marked effect of the bird's age on the worsening of locomotor injuries, which was expected, since the older the bird, the greater its size and weight. The genetic breeding of these animals for years has aimed at the high deposition of meat in a short time, accelerating the growth rate. Moreover, the inclusion of *Zoysia japonica* grass as an alternative material, diluted in the most used materials (shavings and rice husks), positively influenced the maintenance of the integrity of birds' legs for up to 28 days, after 28 days, the inclusion of grasses in both materials negatively affected bird's locomotion.

Evaluating the results of the present study was possible to demonstrate the need to use a bedding material that is able to mitigate the effects of the development and age of birds, in this sense, Knowles *et al*. (2008) could demonstrate the possibility of improving walking ability in accelerated weight gain broilers through alterations in husbandry practice related to flock density and quality of the litter. Bailie and O'Connell (2014) observed a significant decrease in locomotion in older chickens and attributed it to the negative effects of weight gain on leg health, which was reflected in a significant increase in gait and latency to lie scores according to bird age during the experimental weeks.

When analyzing pododermatitis up to 28 days it was possible to include 50% of grass to any bedding material (shavings or rice husks), due to lower pododermatitis scores compared to the inclusion of 25% or without the inclusion of material, evidencing that treatments with 50% grasses were superior to the others.

However, after 28 days, the inclusion of grasses in both materials negatively affected pododermatitis scores. One hypothesis, based on studies by Bailie and O'Connell (2014) is that animal weight is not high at the initial stages and volume of excreta is low, consequently with advancing bird's age greater is the bedding moisture and compaction.

The development and severity of pododermatitis can vary significantly according to the material used and it is related to the higher bedding moisture content, as described by Toppel *et al*. (2019). The moisture increases along with the time the birds stay on the bedding, negatively affecting the feet health. In this sense, moisture softens and opens the collagen matrix of the cushion skin (Youssef *et al*., 2011), consequently, it would trigger an immune system reaction and stimulates the proliferation of bacteria causing dermatitis (Toppel *et al*., 2019).

With a greater grass inclusion after 35 days of bird age, the characteristics of the litter became compromised making birds more susceptible to injuries in the footpad. Shepherd and Fairchild (2010) suggested that one of the most important characteristics of litter material is its capacity to retain water and lose water faster due to the increase of excreta production and consequently the increase of the moisture of bedding, which seems not to have been achieved with the higher grass inclusions in both bedding substrates.

Regarding the different substrates, the results presented by pododermatitis evaluations suggest that shavings caused higher lesion scores which may be related to the material physical shape as Youssef *et al*. (2010) described, the physical shape can cause skin irritations with the presence of redness if it contains sharp or protruding edges (such as occurs in shavings). This irritation can be considered the beginning of lesions, which get worse as the birds became older, and the litter moister.

Evaluating both legs for *valgus-varus*, the right leg suffered an isolated effect of grass and age and left leg presented interaction of bedding and age, suggesting that this angular deformity is bilateral. V*algus-varus* is generally unilateral, as described by Guo *et al*. (2019), where 82.4% of the cases were unilateral. The age effect isolated or interacting with litter material can be explained by weight gain and consequently the absorption of the impact of the bird's steps on the bedding. Over the weeks, the birds practicing their moving behavior, placed greater weight on the legs, aggravating the angular deformity regardless of the material used (rice husks or shavings), demonstrating that the impact absorption capacity of different materials had little or no effect on the development of this injury under the conditions of the present study.

Regarding the incidence of tibial dyschondroplasia, any effect of the treatments was observed. This suggests that the grass inclusions in the bedding materials do not interfere with the occurrence nor in the development of this pathology. Edwards (1984), reported that one of the causes for the occurrence of tibial dyschondroplasia is low levels of calcium and high levels of phosphorus in the diet. This helps to explain non-significant differences in scores for these injuries since all treatments received the same balanced diet.

Our results presented higher scores of femoral degenerations in the left leg of treatments with the inclusion of 50% of grass in rice husks. Accelerated weight gain in chickens is a contributing factor for its pathogenesis and the prevalence of leg problems in heavy chickens (Packialakshmi *et al*., 2015). This fact combined with angular injuries may justify the results found here, over the weeks, the birds gained weight, and, in the presence of *valgus* and *varus* angular degeneration, also was observed more severe femoral degeneration in the left leg. There is not an accurate reason for the greater occurrence of the lesion in left or right legs, studies by Dinev (2014) and Kanakov *et al*. (2019) found a majority of chickens were characterized by unilateral abnormality, but neither of them evaluated the ratio left/right. The general cause of leg abnormalities in growing skeletal structure is not able to support the fast increase in body weight; thus, aggravating the femoral degeneration scores (Kanakov *et al*., 2019).

Regarding the bedding material, Thorp *et al*. (2007) reported that environmental factors, such as moisture and bedding quality, play an important role in the development of femoral degeneration. The authors reported that these lesions are often related to focal bacterial infections, such as coagulase-positive *Staphlococci*, coagulase-negative *Staphlococci*, coliform, and mixed bacterial populations, thus the lower quality litter can facilitate the contact of pathogenic agents with the bird's skin and consequently promote the colonization of other structures.

Spondylolisthesis is the most prevalent axial skeletal pathology in commercial chickens and may cause clinical sings as lameness. Research has suggested that broilers with a lower degree of skeletal maturity are more prone to this type of injury (Dinev, 2014). Despite that, in this study, no effects were found regarding the litter material or grass inclusion in spondylolisthesis occurrence, which suggests the lameness found in the birds possibly were not related to lesions in the spine but related to disorder in the legs and feet of birds.

Changes in skeletal morphology are the result of changes in bone length, weight, width, and curvature. Guo *et al*. (2019) reported that bone indexes for the femur, tibia, and metatarsus were higher in birds affected with *valgus* and *varus* angular deformity. In the present study, significant results were not observed in the analysis of Seedor index and resistance to breakage of tibial bones, even in the presence of angular degenerations.

Our results indicated that dried grass inclusion in broiler bedding is in the production of "griller" chicken, which means chickens that were slaughtered younger and lighter. Conventional chickens are produced in Brazil in cycles of 42 to 45 days, weighing from 2.4 kg to 2.8 kg at the time of slaughter. The "griller" chicken remains alive for 25 to 30 days, and is slaughtered between with an average weight of 1.2 kg and 1.5 kg. Griller chicken produced in Brazil is exported mainly to countries in the Middle East and Europe (Iguma *et al*., 2016) and has significant participation in the country's economy. In this sense, it is important to highlight the characteristics of fewer days in bedding, less weight, and early slaughter of the griller chicken, thus, grass inclusions in shavings as bedding for this category are viable for one batch, as the bedding has not been tested for reuse.

The inclusion of grass *Zoysia japonica* negatively affects the occurrence of locomotion injuries after 35 days of bird age.

The limitation of grass inclusion mixed with rice husk is related to a more accentuated presence of pododermatitis. Based on the findings it is recommended to use 25% inclusions of dried grass (*Zoysia japonica*) as bedding to shavings only for early chicken.

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Conflict of interest

The authors declare no conflicts of interest.

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