DELIVERABLE

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DELIVERABLE: DL.3.2.1 – REPORT OF THE STUDY IN EXPERIMENTAL FACILITIES TO ASCERTAIN DIFFERENT INDICATORS AND METHODS FOR BROILER CHICKEN WELFARE ASSESSMENT UNDER DIFFERENT HOUSING CONDITIONS.
ACTIVITY: 3. SCIENTIFIC AND TECHNICAL STUDIES **SUB-ACTIVITY: 3.2. – Scientific and technical studies to validate indicators and methods** **NATURE**: Report **DUE DELIVERY DATE:** 30 September 2021 **PREPARED BY:** ANSES, reviewed by ANIS

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Activity 3. Scientific and Technical Studies

Article 96 (d): Carrying out scientific and technical studies on the welfare of animals used for commercial or scientific purposes.

Sub-activity 3.2: Scientific and technical studies to validate indicators and methods

Objectives:

1. To help the development of indicators and methods for welfare assessment concerning the four priority areas.
2. To address some negative welfare aspects identified, in order to provide technical solutions to improve animal welfare.
3. To answer some queries of the CAs.

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1. Introduction

Fast-growing broiler chickens in conventional rearing systems are commonly associated with welfare problems such as lameness, footpad dermatitis or lack of expression of species specific behaviours. This impairment of welfare is generally linked to fast-growing genetics and to different elements of housing systems and management like high stocking density or baren environment.

Regarding stocking density, broilers must be reared in the EU at a maximum density of 33 kg/m² according to Directive 2007/43/EC (EuropeanCommission, 2007). A derogation can be granted to increase the density to 39 or even 42 kg/m² if further specific requirements are fulfilled (specific documents, thresholds for some environmental parameters,…). In certain countries, like France, broiler farms are very generally granted derogations to increase the stocking density to 39 or 42 kg/m². For example, 82% of French (conventional) chickens are reared at densities between 39 and 42 kg/m² (FCEC, 2017). At European level, 26% of chickens kept for meat production are reared between 39 and 42 kg/m², 40% between 34 and 39 kg/m² and 34% up to 33 Kg/m² (FCEC, 2017).

Enriching the environment could be a way to improve chicken rearing conditions and welfare. According to Newberry (1995), environmental enrichment is a modification of the environment of captive animals, thereby increasing the animal's behavioural possibilities and leading to improvements in the biological function. There are several kinds of enrichment that can be used with broilers like elevated resting-places (such as perches or platforms), bales of substrate or materials to stimulate foraging and dustbathing behaviours, panels and barriers (Riber et al., 2018). Elevated platforms have been the subject of some recent studies. These studies compared platform and perches (Malchow et al., 2019a), different types of platforms and configurations (number, surface, height, materials) (Bailie et al., 2018; Baxter et al., 2020; Malchow et al., 2019b) and studied their use in commercial farms (Kaukonen et al., 2017b) or experimental conditions (with a small number of animals) (Chuppava et al., 2018; Liu et al., 2020; Yang et al., 2019). Elevated platforms seem to be more suitable than perches for fast-growing broiler chickens, because of the broilers' weight, leg weakness or difficulties to find their balance on "traditional' perches like bars (Riber et al., 2018). In those studies, several parameters are evaluated like economics (Jones et al., 2020), health (Pedersen et al., 2020) or animal welfare (Bach et al., 2019; Tahamtani et al., 2020; Tahamtani et al., 2018). The outcomes of these studies are sometimes contradictory (Riber et al., 2018). Some limited animal welfare improvement could be explained by a too low platform surface or a late setting up (after 7 days old) (Bailie et al., 2018), or by a lack of access ramps (Bailie et al., 2018; Baxter et al., 2020).

Straw bales are sometimes used in farms to enrich the environment. This enrichment helps birds express normal behaviour (such as foraging, pecking and perching), at least on slow or moderate-growing genotypes, or at a lower density (Ohara et al., 2015). Animals use them to lie against them (at a young age) when resting and then to perch on them (Bergmann et al., 2017). Riber and colleagues (2018) reviewed the work on these enrichments with studies showing either no effect or contradictory results on slaughter weight, mortality and locomotion of animals. Baxter and colleagues (2017) showed no effect of adding such bales on ammonia levels and litter quality in commercially reared fast-growing chickens and had mixed results on behaviour. For Kells and colleagues (2001) this enrichment had a positive effect on resting/activity, locomotion and grooming behaviours in commercial farming. Bailie and O'Connell (2014) studied the difference in behaviour according to two quantities of straw bales distributed (one bale per 44 m² or 29 m², on Ross and Cobb chickens at 30 kg/m²), but did not observe any differences in behaviours or leg health.

The benefits of providing enrichments in relation to welfare of animals reared at high stocking densities still need to be validated in a controlled and experimental setting, with pens of significant size, especially in regards to fast-growing chickens. As EURCAW-POULTRY-SFA, our aim is also to assess animal welfare with a set of indicators including Animal Based Indicators, usable for the others activities of the Centre (welfare indicators and the associated methods), to acquire knowledge on their use, notably in situations with enriched environments, high stocking density, and fast-growing chickens.

An experiment was designed for the purposes of improving the knowledge on enrichment and broilers welfare and on indicators and methods in a context of enriched pens.
The aim of this experiment was therefore to compare the welfare of the chickens reared: i) at different densities: 41 kg/m² or 31 kg/m² and ii) with or without enrichment: platforms with access ramps and straw bales (these are two types of enrichment that can be found in "innovative" farms or labelled productions).

2. Materials and Methods

2.1. Housing and experimental design

The study was conducted with 14994 Ross 308 broiler chickens reared up to 33 days of age in an experimental building of 6 rooms with 2 separate floor pens in each one.
The experimental design was 2x2 modalities, with three repetitions (3 pens) by modalities: stocking density maximum of 41 kg/m² or 31 kg/m², with or without enrichment. In the original protocol, we intended to compare the densities 33 Kg/m² (maximum density allowed without the derogations by the Directive 2007/43/EC (EuropeanCommission, 2007)) and 40 Kg/m². But, because of differences between the real growth of chickens and what was expected, final stocking densities were different, but still made room for interesting comparisons.
All pens were 72m² (6x12 meters) but space under feeders and platforms were considered as not accessible (since animals could not access these areas all the time), thus as not useable area. Only the useable areas were used to calculate the number of animals per m² to respect the final density for each modality. In particular, we hypothesized that animals could not access the surface under the platforms during the last part of the rearing stage due to increase in body size (platforms were at 30 cm high). The surface on the top of the platforms was not counted as useable area because it was not a littered area to be in accordance with the European regulation (EuropeanCommission,

2007). On the other hand, the surface on the top of straw bales was counted as useable area since animals could access it and the flooring can be considered as litter. Without enrichment, pens were then considered 70 m² of useable area and 66 m² with enrichment. The usable area was used to calculate the number of chicks placed in each pen (Table 1).

In all pens, the bedding material was 1 Kg/m² of wood shaving litter with addition of litter to maintain an acceptable condition when necessary. In each pen, there were three lines of 29 nipple drinkers, and 16 circular feeders. Animals had natural light. The shutters were opened from 7 am to 8 pm. During the first week of age, chicks were exposed to a lighting program of 23L:1D. Then, artificial light was on from 5 am to 11 pm from one week of age.

Table 1: Distribution of animals per modality in the 12 pens of the six rooms of the experimental building

Density	41 kg/m²						31 kg/m²					
Room	*Room 1*		*Room 2*		*Room 3*		*Room 4*		*Room 5*		*Room 6*	
Enrichment	Yes		No		Yes	No	Yes	No	No		Yes	
Pen	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*
Number of animals	1385	1385	1471	1471	1385	1471	1040	1103	1103	1103	1040	1040

2.2. Enrichments

Platform

A platform equipped with two access ramps were placed in the middle of each enriched pen (Figure 1). The platforms were made of plastic slatted floor, 30 centimetres high, two metres long and one metre wide. The access ramps placed on either side of the platform had a 16° slope and measured 1m x 1m. The total surface area of the platform was therefore 4 m² with 3 m² potentially usable underneath, although not counted in the usable area. Platforms were available for animals from the first day of life to the last day.

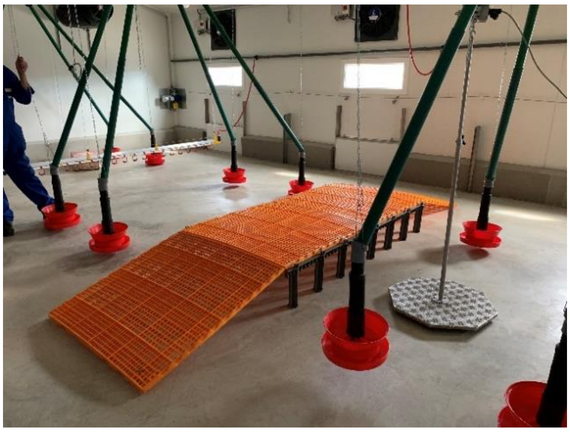

Figure 1: Photograph of one platform used in enriched pens

Straw bales

Two straw bales were placed on each side of the barn. They were 80 centimetres long, 40 centimetres wide and 19 centimetres high (2 x 0.32 m² per pen). They weighed around 10 kg and were removed from their plastic packaging beforehand and tied up to ensure a better hold. They were not renewed once disintegrated.

2.3. Measurements

Air quality

Two times a week, NH_3 and CO_2 levels were recorded in a central area of every pen with KIMO portative devices (CO_2: *KIMO AQ 110* and NH_3: *KIMO µGAZTOX*) and above and underneath the platform in enriched pens. Those measures above and underneath the platform were used to check and refine, if needed, the protocol of gas assessment in enriched environments. We wanted to test if gas concentrations were higher above or underneath the platforms than in the rest of the pens.

Besides, CO_2 was automatically recorded with Tuffigo Rapidex device "*Senso Gaz CO_2*" every 15 minutes in each room in order to check the compliance with the previous measurements.

The dust was assessed with the dust sheet test (WelfareQuality®, 2009). A black A4 size paper was disposed in each room at bird height in the middle of the room, between the two pens, twice a week. Then, the sheets were removed after 3 hours and the dust level found on the papers was classified as follows: scores 0: "None", 1: "Little", 2: "Thin covering", 3: "Lot of dust" and 4: "Paper colour not visible". We wanted to check if the presence of enrichment would increase the dust level.

Litter quality

Litter was collected once a week in order to assess the humidity level. A handful of litter (on the surface of the ground, around 10 cm in width, length and depth), was collected in four areas (between feeders) and, in pens with enrichment, underneath the platform. For each pen, the samples of bedding from the four areas (between feeders, not underneath the platforms) were manually mixed to ensure two representative samples. These two samples and the one from the litter collected underneath the platform were weighed, dried for 24 hours at 70°C and reweighed to measure the dry matter (McLean et al., 2002). These measures were done to assess litter humidity level on the surface (where broilers are in contact with) and to compare these results with litter quality assessment protocols that could be used during inspection.

At 28 days of age, the litter quality was evaluated visually by two different observers without talking to each other to assess their reliability[1], using the Classyfarm protocol (Vinco et al., 2020) and the Welfare Quality Protocol (WelfareQuality®, 2009) to compare these two scoring systems. Both scoring systems were done in three areas of one square meter per pen (same areas chosen in each pen): under the drinking line, in the middle of the pen and between the feeders. With the Welfare Quality protocol, the litter was scored on a scale of 0 to 4 where 0= completely dry and flaky i.e. moves easily with the foot, 1= dry but not easy to move with foot, 2= leaves imprint of foot and will form a ball if compacted, but ball does not stay together well, 3= sticks to boots and sticks readily

[1] Reliability: The extent to which results are largely the same when the same observer repeats assessments after receiving reasonable training or the agreement between two or more observers after they have received reasonable training.

in a ball if compacted, 4= sticks to boots once the cap or compacted crust is broken. With the Classyfarm protocol, a score describing the wetness and the friability of the bedding material (1-10 scale) was given for each area (Table 2). The mean of the friability and wetness scores was calculated to have the general score of Classyfarm.

Table 2: Classyfarm litter quality assessment scale (Vinco et al., 2020)

Score	Friability Description	Wetness Description
1	Completely caked	Wet litter, water is appearing by pressure on the litter of the total area
2	80-90 % area caked	Wet litter, water is appearing by pressure on the litter beneath drinkers
3	70-80 % area caked	Wet litter, no water is appearing by pressure on the litter
4	60-70 % area caked	Wet litter dark coloured. Litter can be pressed into ball-shape
5	50-60 % area caked	Wet litter, dark coloured. Larger ridges beneath drinkers
6	40 % area caked	Almost dry litter, small ridges beneath drinkers. Litter between drinkers and feeders is still friable
7	30 % area caked	Almost dry litter, dark coloured beneath drinkers and in other areas light coloured, ridge formation just started beneath drinkers
8	10 % area caked	Almost dry litter, light coloured, no ridges beneath drinkers
9	Friable litter, small caked areas	Dry litter, light coloured
10	Friable litter, no caked areas	Very dry litter (only observed at start)

Use of the enrichments

During the rearing period, direct observations were done at 4, 7, 12, 15, 18, 21, 26, 28 and 32 days of age in order to record the use of enrichments by the animals. Concerning the platforms, the number of animals perching on it and its access ramps was recorded. About straw bales, we observed the number of animals that was interacting with each straw bale in three types of ways already noticed in literature (Kells et al., 2001): pecking the straw bale, clustering around it and standing on top of it. Because of a disintegration of bales, notably by the weight of animals standing on the top of them, we were not able to record the animals' interactions with straw bales after the 21st day of age, that is to say the three last observations dates.

In addition, videos of animal behaviour undisturbed by human presence were obtained from camera recordings in two enriched pens (one per stocking density) from 0 to 16 days of age (videos after that day were not exploitable due to technical constraints). Observations were done at six time points of each day (5am, 7am, 12 am, 2pm, 5pm and 7pm). As for the direct observations, the use of enrichment by the animals was recorded (perching on the platform, clustering around and standing on top of each straw bale) to check the compliance with direct observations.

Lameness

To assess lameness, lame score was noted at 26 days and 32 days on 20 randomly chosen birds in each pen. The observer walked toward one bird at a time. Birds either moved independently or were encouraged to walk, stimulating them by voice or gently with the foot or hand. Scores were assigned using a 0-3 scale inspired from Meyer et al. (2020) where 0 = ability to walk with no signs of lameness, 1= unevenness in steps or stopped and sat down but ability to walk 1.5m, 2= severe disability, birds can walk a few steps but not 1.5 m and 3= bird unable to walk.

Weighing, mortality and Body Condition

Every day during the rearing period, mortality was calculated and the average weight of animals was automatically recorded in each pen with an automatic weighing scale.

On days 25 and 32, 50 sexed animals per pen (half males and half females) were taken randomly for weighing and evaluation of footpad dermatitis and hock burns.

- Footpad dermatitis (FPD): Both feet were looked at and the worst was scored. When feet were dirty, they were gently brushed with a toothbrush and soapy water. The scoring system was adapted from the Welfare Quality Protocol (2009). 3 points scoring system: a= no evidence of footpad dermatitis, b= minimal evidence of footpad dermatitis (mild lesions), c=evidence of footpad dermatitis (severe lesions). The distinction between mild and severe lesions were done on the size and the depth of the lesions.

- Hock burns: Both hocks were looked at and the worst were scored. When hocks were dirty, they were gently brushed with a toothbrush and soapy water. The scoring system was adapted from the Welfare Quality Protocol (2009). 3 points scoring system: a= no evidence of hock burn, b= minimal evidence of hock burn, c=evidence of hock burn. The distinction between mild and severe lesions were done on the size and the depth of the lesions.

Post-mortem

At slaughterhouse, birds' footpad dermatitis was evaluated on the whole batch with a camera system detection in three scores (Meyn© footpad inspection system): absence of lesions (score a), middle/minor lesions (score b) and severe footpad dermatitis (score c). This system is supposed to analyse the entire batch but, in reality, 75-95% of the pads are examined (incorrect positioning of the feet, error, etc.).

In addition, one observer have done direct observations on the slaughter line. For each modality, carcasses were observed on the line after bleeding for 15 minutes, to score hock burns. It represented 1850 carcasses observed per modality (speed of the line: 7400 carcasses per hour).

3. Results and interpretation of the results

3.1. Air quality

During all the rearing period, the carbon dioxide and ammonia concentrations were never above the directive 2007/43 limits (EuropeanCommission, 2007) that are 3000 ppm for carbon dioxide and 20 ppm for ammonia. In our study, ranges were 1135-1901 ppm for CO_2 and 4.33-9 ppm for NH_3. The small fluctuations in gas concentrations over time could be a consequence of ventilation variations (Figures 2 and 3)

Through the full period, there was no difference of carbon dioxide or ammonia concentrations between our treatments (Figures 2 and 3). Nevertheless, at 28 days there was a difference of carbon dioxide concentrations between all our treatments (p=0.02) but this difference disappeared when we analysed the data with pairwise comparisons.

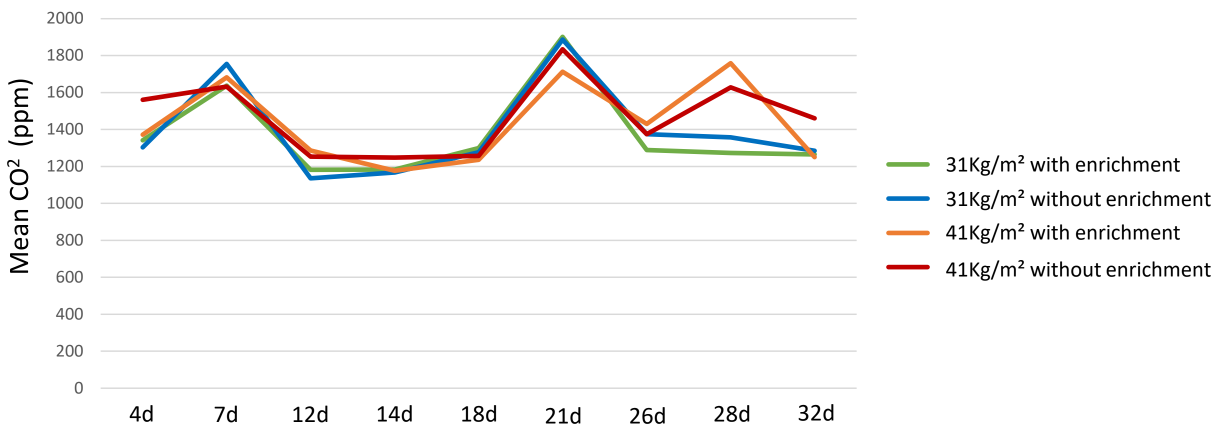

Figure 2: Evolution of mean CO_2 (recorded with portative device) in each treatment per day of age

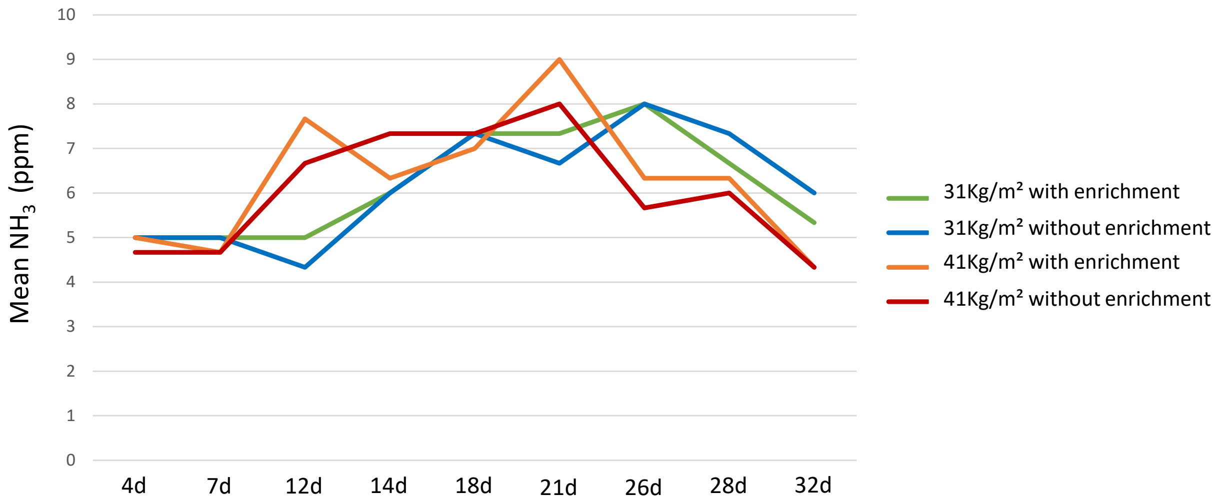

Figure 3: Evolution of mean NH_3 (recorded with portative device) in each treatment per day of age

Measures done with portative devices several times all along the rearing period did not show any significant difference in gas concentrations between areas above or underneath the platforms and in the rest of the pens. In enriched pens with a higher density, there were no difference of carbon dioxide concentrations between the central area of the pen (mean CO_2 concentration = 1434 ppm) and underneath (mean CO_2 concentration = 1481 ppm) ($p=0.09$) or above the platform (mean CO_2 concentration = 1472 ppm) ($p=0.11$). Nor was in enriched pens with the lower density: central area (mean CO_2 concentration = 1374 ppm) vs. underneath the platform (mean CO_2 concentration = 1400 ppm) (p-value = 0.28); central area vs above the platform (mean CO_2 concentration = 1391 ppm) ($p=0.26$). The CO_2 concentration was not significantly different between underneath and above the platform in both densities ($p=0.47$). There was no difference of ammonia concentrations between the central area (mean NH_3 concentrations 31 Kg/m² = 6.2 ppm; 41 Kg/m² = 6.3 ppm) and underneath the platform (mean NH_3 concentrations 31 Kg/m² = 6.4 ppm; 41 Kg/m² = 6.3 ppm) ($p=0.63$), between the central area and above the platform (mean NH_3 concentrations 31 Kg/m² = 6.3 ppm; 41 Kg/m² = 6.1 ppm) ($p=0.71$) or between underneath and above platforms ($p=0.12$) in both enriched treatments. Thus, use of platforms by the animals did not impact gas concentrations in our barns.

Ammonia levels depend on many factors such as the litter type and quality (e.g. pH, humidity), management of water drinkers, air temperature, rearing systems and amount and management of manure, etc. (e.g. (Kristensen and Wathes, 2000; Brouček and Čermák, 2015; David et al., 2015a)). In our experiment, we found no effect of stocking density or enrichment on ammonia levels. Similarly, Baxter et al. (2017) and Kaukonen et al. (2017a) have shown that neither straw bales nor elevated platforms impacted ammonia levels. However, Yang et al. (2019) found lower ammonia levels (up to 27% lower from the last weeks of the rearing period) in broiler pens with elevated platforms than in pens without. Nevertheless, in Yang' study, there were manure catchers (manual cleaning three times every week) under the platforms. Yang et al. conclude that manure catchers were crucial to improve air quality in broilers' environment. In addition, in their study, the ammonia concentrations at the end of the rearing period were largely higher than ours (around 60 ppm in their rooms without elevated platforms and around 45 ppm in their rooms with platforms while ammonia concentrations were never higher than 9 ppm in our study. Besides, ammonia level could also be impacted by stocking density in poultry productions (e.g. (Mendes et al., 2010; David et al., 2015b)), this was not confirmed in our study but it should be noted that in our experimental facilities (experimental modern building), the ventilation system is highly efficient and we did not have any air quality issues.

In previous studies, carbon dioxide levels have never been evaluated according to enriched environment in poultry barn. Like ammonia, no difference in carbon dioxide levels was found between our treatments, showing no effect of density or enrichment. However, in our experimental facilities, we did not have any air quality issues.

Comparing the manual CO_2 measures and the automated ones, we found no significant difference (p=0,13). Hence, our protocol seems valid. Since there is no difference between our gas measurements under the platform and above the platform or in the rest of the pen, there is no need to specifically measure carbon dioxide and ammonia concentrations underneath or above the platform. Nevertheless, gas measurement around platforms should be checked in another type of farm with other ventilation systems.

Concerning dust measurement, all rooms had the same score on each day of test but the score slowly increased with time from 0 the first week of age, to 2 for the rest of the rearing period, regardless the treatment.

Thus, the presence of enrichments did not impact the gas concentrations or dust level in our experimental facilities.

3.2. Litter Quality

Our litter was quite humid with mean litter moisture between 32.46 and 38.06% (Table 3 and 4), despite the regular additions of litter. By comparison, in other studies analysing the litter's humidity in enriched environment, lower values were found: between 26.08 and 28.73% in Baxter et al. (2017), between 30.8 and 32.3% in Bailie et al. (2018) or between 18.56 and 22.02% in Yang et al. (2019). Decreases in the percentages of litter's humidity observed from one week to the next are due to additions of wood shavings to maintain the litter in an acceptable condition.

There was no effect of the density on the litter's humidity content under the platforms (p=0.91) and no effect of density nor the enrichment in the rest of the pens (p=0.71) (Tables 3 and 4). This is in accordance with previous studies on enriched environment, where litter moisture and quality were not impacted by the enrichment (perches, elevated platforms, straw bales and dust bathing substrate) (Baxter et al., 2017; Bailie et al., 2018; Kaukonen et al., 2017a). One exception is the study of Yang et al. (2019) where there was a positive effect of elevated platforms decreasing the litter moisture, but these platforms had manure catcher, which was not our case.

Table 3: Mean percentages of humidity in the litter (3 pens per treatment) in the pens

Age of birds (weeks)	**31 Kg/m² with enrichment**	**31 Kg/m² without enrichment**	**41 Kg/m² with enrichment**	**41 Kg/m² without enrichment**
1	25	19.33	27.33	28.33
2	30.33	30.33	26.66	25.66
3	35.66	26	35.66	22
4	48	45	51	42.66
5	43.66	41.66	49.66	52.66
Mean values	36.53	32.46	38.06	34.26

Table 4: Mean percentages of humidity in the litter (3 pens per treatment) under the platforms in enriched pens.

Age of birds (weeks)	**31 Kg/m² with enrichment**	**41 Kg/m² with enrichment**
1	36.33	41.33
2	53.33	58
3	48.33	46.33
4	54.66	53.33
5	53	48.33
Mean values	49.13	49.46

The litter was more humid under the platform than in the other areas of the pen, whatever the density (31 Kg/m²: p-value=0.0001 and 41 Kg/m²: p=0.005). As a reminder, space under the platform was not counted as useable area because we cannot be assured that the animals would have access to this space during all their growing phase. Nevertheless, we observed that the space under platform was continuously occupied by the birds and litter was not renewed at this location for logistical reasons, as farmers would probably do in commercial situations, since this area is difficult to reach. Furthermore, droppings of animals staying on the top of the platform fell under the platform. However, animals backs in enriched pens were not dirtier than those in the non-enriched pens (personal observation), so droppings probably did not soil more the animals staying under the platform. Thus, it can explain why the litter under the platform was more humid than in the other area of the pen (accumulating faeces from birds above and below the platform).

Scoring of the litter were statistically reliable[2] between the two observers, both for Classyfarm (*humidity score*: r=0.74, p-value<0.0001; *friability score*: r=0.82, p-value<0.0001; *total score*: r=0.83, p<0.0001) and Welfare Quality (r=0.68, p<0.0001) scoring systems. In addition, both litter scoring systems were correlated (r=0.81, p <0.0001).

Finally, we compared our litter quality notation (with these two scoring systems at 28 days of age) with the level of humidity measured in the litter three days before (25 days of age) (different days due to practical constraints). We did not find statistical correlations between the level of humidity

[2] Reliability: The extent to which results are largely the same when the same observer repeats assessments after receiving reasonable training or the agreement between two or more observers after they have received reasonable training.

and the Welfare Quality score but a statistical tendency was noticed (r=0.50, p =0.09). In contrast, the Classyfarm score (mean of humidity and friability scores) was correlated with the percentage of humidity of the litter (cor=0.61, p=0.03). One difference between the two visual litter quality evaluations is that the Classyfarm system has two scores, one of friability and one of humidity, whereas Welfare Quality only has one score combining these aspects. Actually, Classyfarm humidity score was not correlated (but a statistical tendency was noticed) with the established level of humidity (cor=0.51, p=0.09) but the Classyfarm friability score was correlated (cor=0.62, p=0.03). Anyhow, Classyfarm scoring system seems to be more valid than the Welfare Quality scoring system to assess litter's quality and, litter's humidity in particular. However, it could be interesting to explore more the relation between the percentage of humidity measured in the litter and these scoring systems because our protocol did not allow us to do all these observations the same day. Furthermore, litter addition has been done, in the higher density pens, four and five days before the litter collect for humidity assessment. Thus, we can assume that the quality of the litter was better when it was collected in these pens (four and five days after litter addition) than when it was scored (six days after litter addition). One can speculate whether the litter humidity had been assessed the same day as the scoring, both of the scoring systems would have been correlated with the humidity level.

The reliability between two observers was confirmed for both tested methods (Welfare Quality and Classyfarm) but it needs to be tested with more observers before any definitive conclusion. The validity of these two methods was tested by correlation with lab analysis of the humidity level of the litter. Classyfarm's protocol seemed to be more valid than the Welfare Quality protocol because it was the only one correlate to the humidity level of the litter but, because of practical constraints, this result needs more investigations.

3.3. Weight and Mortality

In our experiment, there was no effect of the enrichment on the body weight assessed manually (p=0.06) (Table 5) or with weighing scales (p=0.60). The stocking density neither influenced the body weight assessed manually (p =0.10) or with weighing scales (p =0.82).

Mortality (Table 5) was not affected by stocking density (p-value=0.66) nor enrichment (p-value=0.64). The cumulative daily mortality rate of three treatments was above the calculated legal rate[3] which was 2.92% in our case. Only the group of *41 Kg/m² without enrichment* was slightly under this legal rate with a cumulative daily mortality rate of 2.90%. Our mortality rates were affected by a high number of dead animals during the six first days in one pen (on a total of three pens) in two treatments (one pens in *31 Kg/m² without enrichment* and one pen in *41 Kg/m² with enrichment*). These early deaths were not linked to the density (very low in young animals), nor

[3] Council Directive 2007/43/EC, Annex V on the criteria for the use of increased stocking density: "in at least seven consecutive, subsequently checked flocks from a house the cumulative daily mortality rate was below 1 % + 0,06 % multiplied by the slaughter age of the flock in days."

enrichments (no animal was found injured due to any enrichment), and could be explained by external problems from the hatchery or the transport conditions of chicks, but our investigations were unsuccessful.

Table 5: Percentage of cumulated mortality at the end of the rearing period and means of body weights at days 25 and 32 days (manually assessed) according to treatments

		31 Kg/m² with enrichment	**31 Kg/m² without enrichment**	**41 Kg/m² with enrichment**	**41 Kg/m² without enrichment**
Cumulative daily mortality rate (found dead and culled)		5.2%	5.8%	4.6%	2.9%
Mean body weight (g)	*25 days*	1376 g	1357 g	1350 g	1314 g
	32 days	2074 g	2032 g	2047 g	2011 g

Our results showing no effect of the enrichment on the mortality and weight are not different from what was already found in literature on the same genotype (Ross 308) with different types of enrichment (straw bales, various shapes and height of perches and platforms) whether on commercial farms or experimental field (Baxter et al., 2017; Bailie et al., 2018; Malchow et al., 2019b; Baxter et al., 2020) except for two studies with opposite results. Indeed, Ohara et al. (2015) found a greater mean final body weight for enriched pens (straw bales and perches) than control (males: 3100 g in enriched group vs. 2960 g in control group; females: 2450 g in enriched group vs. 2390 g in control group) on Tatsuno slow-growing broilers (slaughtered at 60 days). Moreover, they found a higher mortality in males and a lower in females in enriched pens than in control (males: 11.8% in enriched group vs. 9.6% in control group; females: 5.2% in enriched group vs. 9.4% in control group), but in our study no distinction of sex was made when rating the mortality. De Jong et al. (2021) have found that broilers (males from two strains: Ross 308 slaughtered at 38 days and slower growing JA757 slaughtered at 53 days) reared without enrichment were heavier from day 17 and onwards than birds reared with enrichment. However, mortality did not differ significantly (varying from 4.8 to 5.7%). In this recent study of De Jong et al (2021), pens were very small (3m²) and with several enrichments (barrier perches, platform with ramps and dustbathing area), thus quite different from ours. These authors found that enrichment increased activity of birds and then have adverse effects on performance (average body weight and other parameters). This conclusion differs from Ohara et al. (2015) who observed an increased activity in enriched environment but deduced that enhancing broilers activity with enrichments may not have adverse effects on productivity since they found a greater average body weight in enriched pens.

3.4. Use of the enrichments

Direct observations

Only descriptive statistics were done on data from the next behaviours' direct observations. Indeed, these observations should be consider as preliminary work in order to orient futures researches.

In the following figures (Figure 4 to Figure 8), we set out the mean number of broilers observed in three pens of both stocking densities (pecking the straw bales, laid against the straw bales, perched on the straw bales, present on the access ramps of the platform or perched on the platform) per day of observation. The observations of the birds' interactions with the straw bales have stopped at 21 days of age after disintegration of the bales.

Pecking the bales:

Animals reared at the lower stocking density seem to have pecked at balls more than those from the highest density from day 4 to day 12 while birds reared at the highest density pecked more after 15 days (Figure 4). During the first two weeks, in the lowest density pens there were 2.3 to 6.7 animals pecking by straw bale (representing 0.23% to 0.65% of the animals in pens) while from 15 days of age there were only 1.8 to 2.6 birds pecking (0.18% to 0.26%). In the highest density, there were 2 to 4 birds birds pecking by straw bale from day 4 to day 12 (representing 0.15% to 0.29% of the animals in pens) and 2.3 to 4 after 15 days of age (0.17% to 0.3%). In any case, few animals were observed pecking the straw bales during the observation, thus no conclusion can be done on these data. Further research needs to be done on this subject.

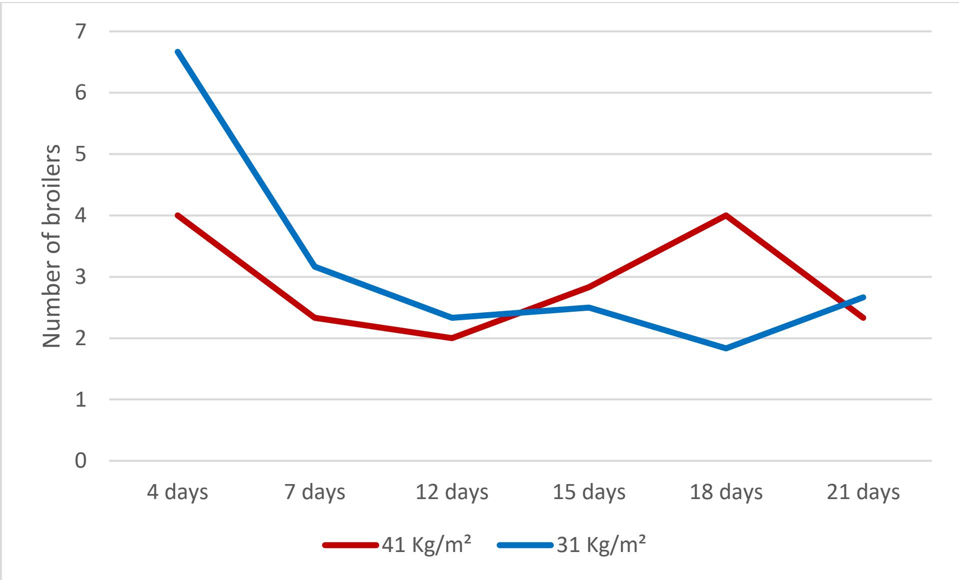

Figure 4: Mean number of broiler chickens pecking a straw bale per density per day of age

Animals laid against and perch over the bales: We observed no difference between the two stocking densities regarding the number of animals laid against the straw bales. There were more animals laid against straw bales during the first week than after (Figures 5). Indeed, during the first week, there were 24.5 to 31.2 birds laid against a bale in average (2.41% to 3% of the animals in pens). Broilers spending more time clustering around straw bales at a younger age had already been observed in previous studies (Kells et al., 2001; Bergmann et al., 2017). Bergmann et al. (2017) named this behaviour observed, decreasing with age, "shelter-seeking huddling". Indeed, this need of cluster around an object like straw bales, or roof support poles in some other case (Kells et al., 2001), is probably explained by a seek for shelters. Kells et al. (2001) also mentioned that birds were clustering around support poles (in houses without straw bales) at all ages even if the most clustering took place when the birds were youngest. We could also explain this decrease in the number of animals laid against straw bales after the first week by their increasing body size (when they are bigger, less animals can be around the straw bales).

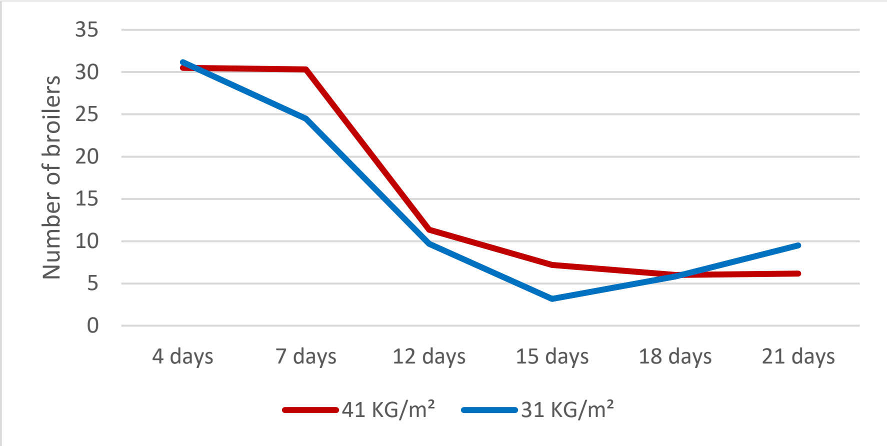

Figure 5: Mean number of broiler chickens laid against a straw bale per density per day of age

Between 7 and 12 days of age, birds started to perch on the straw bales with a mean of 1 bird perched per bale at 7 days of age (Figure 6) until reaching a number of 7.8 to 8.3 birds (on a straw balls of 0.32 m² initial surface) perched at 15 days before a decrease, that might be due to lack of bales compacticity and ability to support animals. Kells et al. (2001) didn't notice an increasing evolution in time of the number of animals standing on the top of straw bales but Bergmann et al. (2017) did as Bach et al. (2019). In Bach's study, fewer animals were standing on the top of straw bales at 6 days of age and then, at day 34, than the rest of the time (at 13, 20 and 27 days of age). Indeed, at a young age, the broilers could have difficulties to reach the top of straw bales to perch and, when they are older (in the last days of the rearing period), the declined locomotor activity of fast-growing strains prevents them to jump on it. Besides, space on the top of the bales did not allow many broilers being perched on the same time since their body size is increasing quickly, this also explaining the decrease of the number of animals standing on the top of straw bales over time.

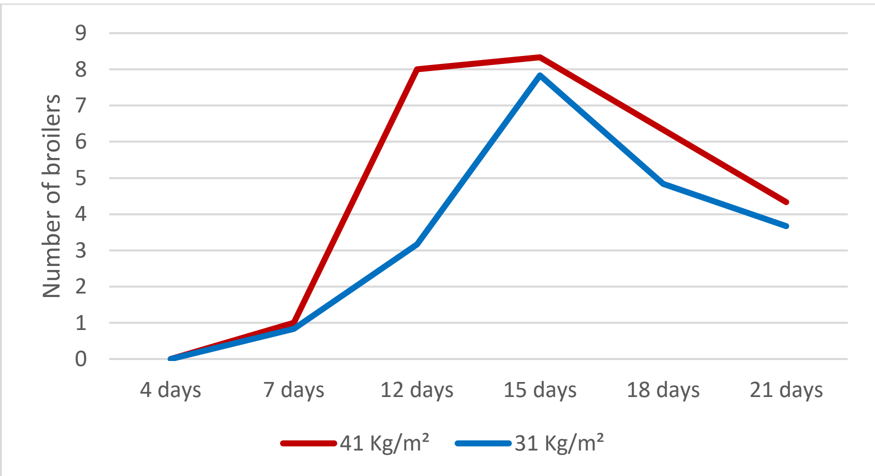

Figure 6: Mean number of broiler chickens perched on a straw bale per density per day of age

Use of the platform: Regarding the use of the platform, we observed that broiler chickens used the platform and its ramps from the first day to the end of the rearing period (Figures 7 and 8) with an increase of the number of birds perched on the platform with age until 12 days of age reaching a number of 42.3 animals perched on the top of the platform (2 m²) in the lowest stocking density (4.19% of the broilers in the pen) and 53 birds in the highest (3.95%) (Figure 8). After 12 days, the number of animals perched on the top of the platform decreased slightly but stay quite stable in the 41 Kg/m² density (30 to 42 animals between 15 and 32 days) and higher than in the 31 Kg/m² density (17.7 to 41.7 animals between 15 and 32 days). Concerning animals staying on the access ramps of the platforms (2 x 1 m²), there were more animals on the ramps in the 41 Kg/m² density (between 13.7 birds - at 28 days- and 45.7 birds–at 12 days-) than in the 31 Kg/m² density (between 8.33 birds –at 4 days- and 26.7 bird –at 7 and 15 days-) but access ramps were occupied all along the rearing period in both densities (Figure 7).

We observed that chicks started to use the platforms from the beginning without problems of animals stuck in the platform's grid holes. Our observations match with those of the study of Kaukonen et al. (2017b). Indeed, the farmers of this study observed that Ross 308 chicks started to use the platforms (30 cm height) immediately when access was allowed, between 3 and 7 days of age, and they estimated that platforms were used between 50 and 100% of the platforms' surface throughout all the rearing period. We also observed that the birds started to occupy the platforms in the first days and at the end of the rearing period, all the platforms were full. Though, in a previous study comparing the use of three types of platforms' height with three genotypes (Ross 308/fast growing broiler strain, Lohmann Dual/medium-growing dual-purpose strain and Lohmann Brown Classic/slow-growing layer strain), it has been found that Ross 308 broilers made very little use of platforms of 30 cm height and preferred platforms of 10 cm height (Malchow et al., 2019b). In our study, we noticed a good use of our 30 cm height platforms but one important difference between these two previous studies is the inclination angle of the ramp. In Malchow et al. (2019b), the angle was 35° against 16° for our access ramps. In Kaukonen et al. (2017b), the platforms of 30 cm height had an inclination angle of the ramps of 14.5° and were well used. Thus, if the inclination angle of the ramp is too steep, the animals would have trouble climbing on the platforms, especially if they have locomotion issues like in fast-growing broiler strains. Then, we recommend being careful with access ramps' angle. Further research needs to be done on this subject.

Ramps were not only used as an access area to the platform because there were animals staying on them, although in general, there were more animals on the platform than staying on the access ramps (see Figures 7 and 8). These findings are similar to those of Bailie et al. (2018) and are linked to the birds' tendency to prefer horizontal to angled perches. As well as straw bales, at the highest stocking density, the number of animals using the platform is likely to be higher due to the higher number of birds reared in the pens. However, there is no other studies to compare with our results, because to our knowledge, this is the first study to investigate the difference of broilers' interactions with platforms depending on the level of stocking density, in contrast to studies on perches (reviewed in (Riber et al., 2018)).

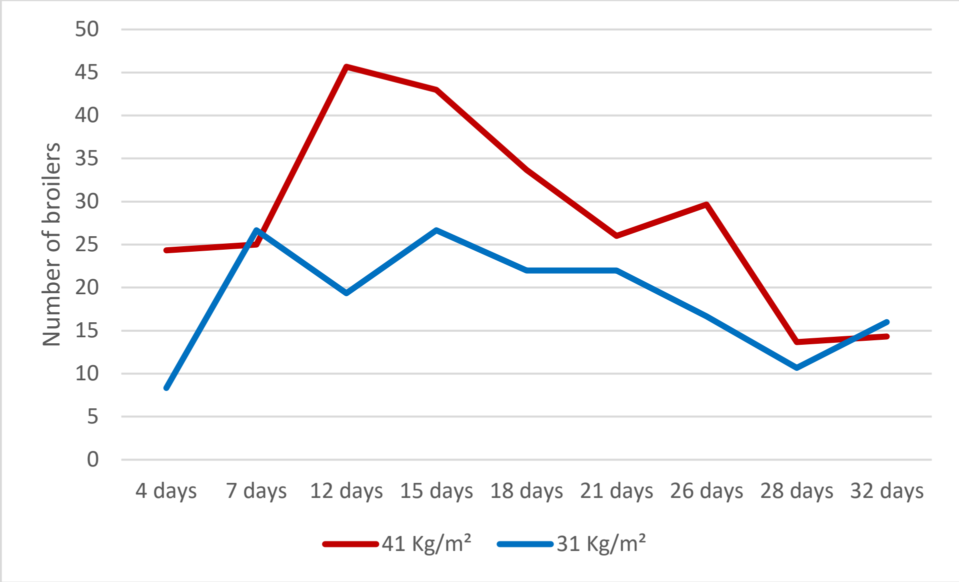

Figure 7: Mean number of broiler chickens present on access ramps of the platform per density per day of age

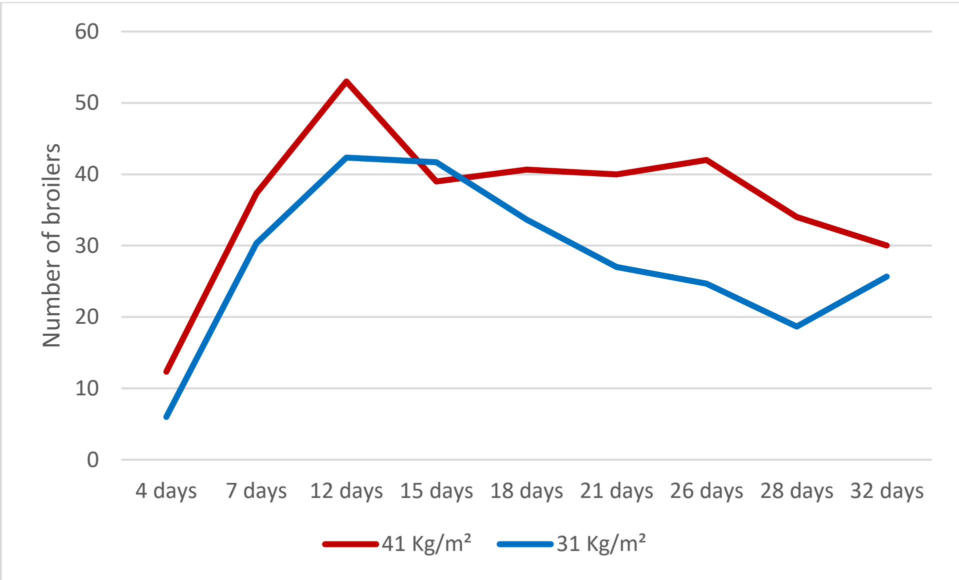

Figure 8: Mean number of broiler chickens perched on the platform per density per day of age

We investigated the difference of density between the platform (top of the platform and access ramps) and in the rest of the pen in order to deepen the birds' motivation to perch on the platform. At 12 days, when the number of animals perched reach a maximum, birds in the highest density group were from 86 to 116 on the platform (top + access ramps) according to the pen; i.e. 21.5 to 29 animals per m² corresponding to a density of 9.9 Kg/m² to 13.3 Kg/m². Meanwhile, the mean density in the rest of the pens was 8.65 Kg/m². In the lowest density group, at 12 days of age, birds were from 44 to 86 on the platform according to the pen; i.e. 10 to 21.5 animals per m² corresponding to a stocking density of 4.6 Kg/m² to 9.9 Kg/m² and the mean density in the rest of the pen was 6.6 Kg/m². For both modality, the densities on the platforms were, almost in all of the pens, higher than in the rest of the pen. However, at 12 days of age, the stocking density is low, therefore not sparsely influencing the animals' behaviour. Thus, these observations highlight the motivation of broilers to have access to elevated structures.

To go further, we investigate this difference of density between the platform and the rest of the pen at the end of the rearing period, where the stocking density reach its maximum. At 32 days, birds in the highest density group were from 37 to 51 on the platform according to the pens (i.e. 9.25 to 12.75 broilers per m²) corresponding to a density of 18.9 Kg/m² to 26.1 Kg/m². In the rest of the pen, the mean density was 36.9 Kg/m². In the lowest density group, birds were from 37 to 47 on the platform according to the pen (i.e. 9.25 to 11.75 animals per m²) corresponding to a density of 19.2 Kg/m² to 24.4 Kg/m². Meanwhile, the mean density in the rest of the pens was 27.1 Kg/m². At 32 days of age, the densities on the platforms were always lower than in the rest of the pens, so we could hypothesize that platforms are used by the animals to escape the higher stocking density. However, at the end of the rearing period, we observed that the platforms were generally full; the lack of space did not allow more animals to perch. Furthermore, this hypothesis does not explain the results on day 12 where the density was generally higher on platforms, showing a real motivation of the animals to perch. Another explanation could be that on platforms, there were more resting animals while in the rest of the pen animals also moved to drink or eat. Thus, animals would climb on the platform to rest quieter.

Use of the space under platforms: During our visits, we also looked at the animals under the platform. The space under the platform was often occupied by the animals (standing position at the beginning of the rearing period and then later in lying position). A hypothesis can be that the area under the platform could act as a shelter for birds and this is why it was highly occupied almost throughout the rearing period, without making the birds of the enriched groups dirtier. In previous studies, the area under platforms was not accessible for broilers because it was fenced off or the platform was not high enough (Bach et al., 2019) or the space under the platform was accessible for broilers only in the last weeks of the rearing period (Baxter et al., 2020). We recommend deepening the knowledge on the use of the space under platforms by the animals in future studies.

Video recording

Based on data from the video recordings (reminder: one pen per stocking density, from 0 to 15 days), there was no effect of density on the number of animals on the platforms and its access ramps (p-value=0.59) or around the straw bales (p-value=0.12). We noticed a rise of the number of animals perched on the platform and its access ramps as in our direct observations, reaching a mean of 64.83 animals perched at 9 days of age in the highest density pens and 45.67 birds at 10 days of age in the lowest density (Figure 9). Considering the number of animals around straw bales, animals were less numerous over time clustering around the bales (Figure 10). During these two weeks of video recording, the highest number of animals around straw bales was observed at one day of age with a mean of 25.42 birds for the 31 Kg/m² density and 35 for the 41 Kg/m² density ($p > 0.05$).

In contrast, there were more broilers in the higher stocking density (41 Kg/m²) pen on the top of the straw bales than in the 31 Kg/m² pen (p-value=0.001). In the 41 Kg/m² density-pen, birds started to perch on the straw bales at 5 days of age until reaching a peak at 8 days with 9.33 broilers perched (mean of 6 daily scans). This was postponed 2 days later in the 31 Kg/m² density-pen, with lower number of animals perched (mean of 6.83 birds) (Figure 11). This difference between densities could have been explained by the motivation of broilers to escape high stocking densities in the pen but since the stocking density is still low at 8 and 10 days of age, it seems unlikely. More investigations are needed since only one pen per density was observed. However, this result confirm our direct observations showing that animals started to perch on straw bales between 4 and 7 days old. These results cannot be compared with literature because, to the authors' knowledge, this is one of the first studies to investigate the differences of broilers' perching behaviour depending on the density.

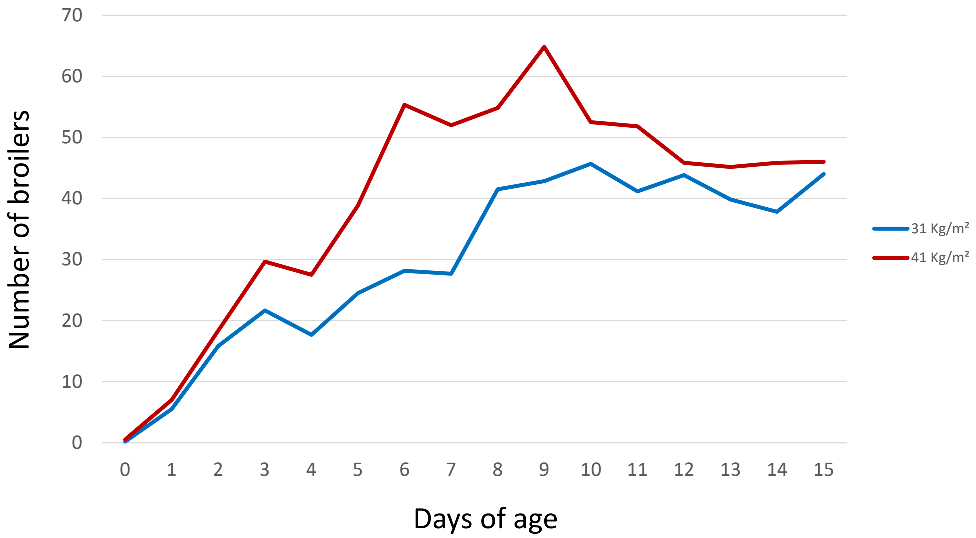

Figure 9: Mean number (6 scans) of birds on the platforms and access ramps per stocking density per days of age

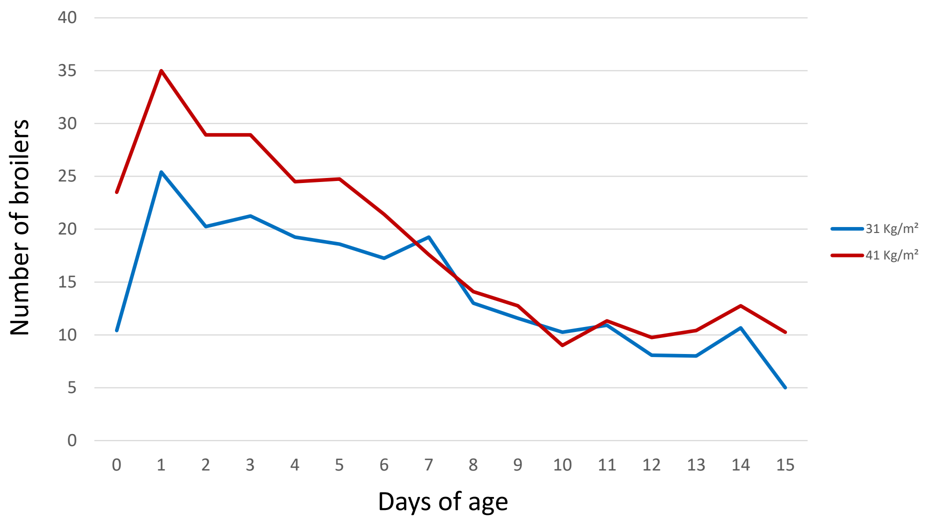

Figure 10: Mean number (6 scans) of birds around the straw bales per stocking density per days of age

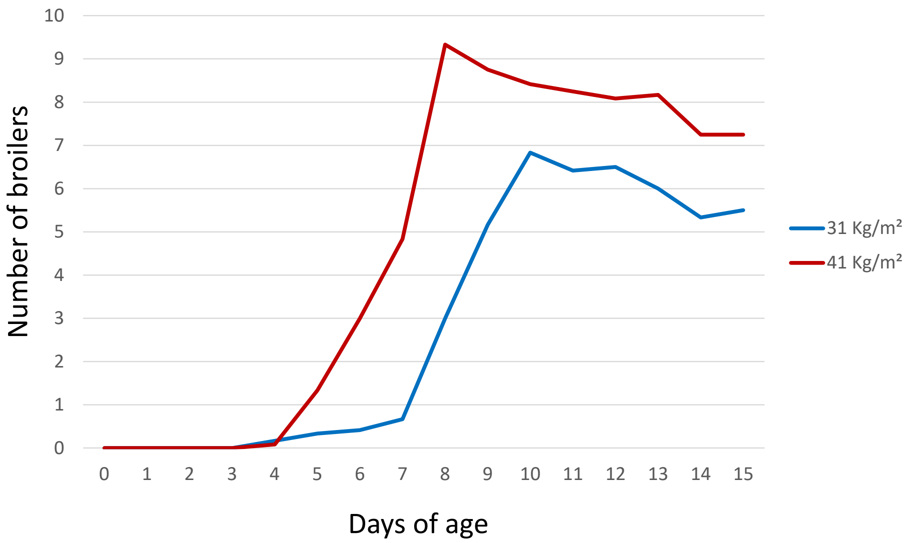

Figure 11: Mean number (6 scans) of birds on the top of the straw bales per stocking density per days of age

Figure 12: Broilers crowding around and staying on the top of straw bales ©Anses

Our behavioural observations must be taken with care. Direct observations were done regularly throughout the rearing period, but only once per day, at the same hour. Behaviour can vary highly from one moment to another (due to external changes for instance), circadian rhythm and the presence of human observing can also influence the behaviour. On the other hand, observations through cameras have been done on the first 2 weeks of age only, due to a technical problem. With camera, the space under platform could not be observed, as well as fine details such as animals pecking bales. Moreover, only one pen per stocking density has been observed with cameras, limiting statistical conclusion. Thus, more research should be done to confirm or disprove these results.

3.5. Lameness

Lameness can be defined by the inability to use one or both limbs in a normal manner. It can vary in severity from reduced ability or inability to bear weight, total immobility (WelfareQuality®, 2009). In our experiment, stocking density impacted walking difficulties at the end of the rearing period: animals reared at the highest stocking density of 41 kg/m² had poorer walking ability than those from the stocking density of 31 Kg/m² at 32 days of age (p=0.05) (Figure 13). This result is consistent with literature showing evidence for a decrease of walking ability when density is increased (e.g.(de Jong et al., 2012)).

Enrichment also had an effect on walking ability as soon as 26 days of age. At 26 days of age, in groups of broilers reared at 41 Kg/m², we observed more animals with walking difficulties in the unenriched group (37%) than in the enriched group (17%) (p=0.02) but this effect disappeared at 32 days. However, at 26 and 32 days, enrichment seemed to reduce negative effects of stocking density. Indeed, for enriched groups, there was no effect of the stocking density on lameness at 26 days (20% of animals with walking difficulties in lower stocking density and 17% in higher stocking density, p=0.81) or 32 days (40% of animals with walking difficulties in lower density and 58% in higher stocking density, p=0.07) whereas we saw that stocking density impact negatively the walking abilities of animals reared without enrichment at 26 days (12% of animals with walking difficulties in lower stocking density and 37% in higher stocking density, p=0.002) and at 32 days (50% of animals with walking difficulties in lower stocking density and 72% in higher stocking density, p=0.02) (Figure 13). Generally, in previous studies on platforms and straw bales, there was no effect of these enrichment materials on the walking ability (Bailie and O'Connell, 2014; Baxter et al., 2017; Kaukonen et al., 2017b; Bailie et al., 2018; Yang et al., 2019; Baxter et al., 2020). However, in all those studies, walking difficulties were rarely observed unlike our study where the number of animals with walking difficulties (scores 1, 2 and 3) was potentially high enough to see an effect of the enrichment. Only the results of Kaukonen et al. (2017b) coincide with ours, showing a positive effect of elevated platforms on the mean gait score of broilers.

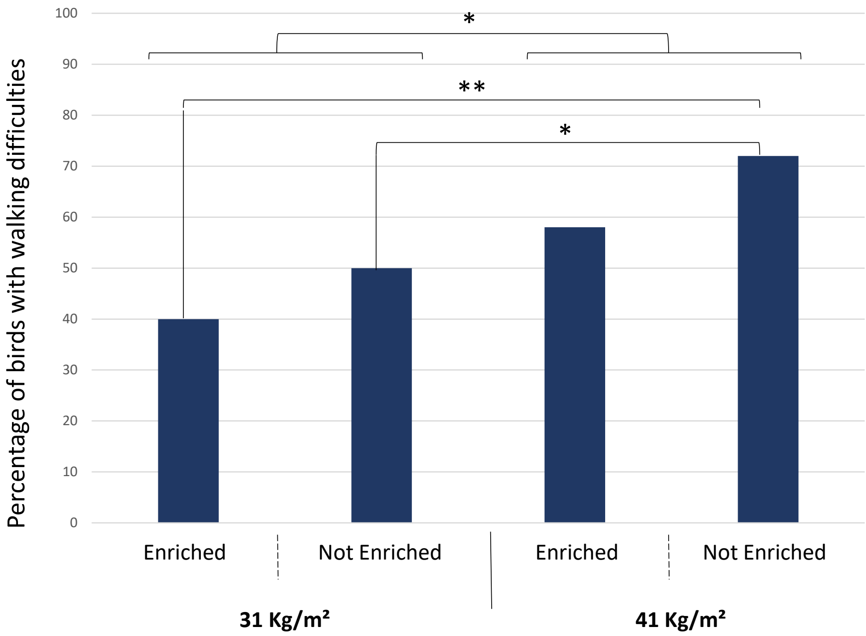

*Figure 13: Percentage of animals showing walking difficulties (score 1, 2 and 3) by modality at 32 days of age. *: p< 0.05; **: p< 0.001*

3.6. Body condition on farm

Footpad Dermatitis (FPD)

Under our experimental conditions, we saw an effect of stocking density on the presence of FPD (Figure 14 and 15). Animals reared at lower density of 31 Kg/m² had less FPD (scores b + c) than those raised at the higher density of 41 Kg/m² at 25 days (31 Kg/m² with enrichment: 90.67% ; 31 Kg/m² without enrichment: 85.4% ; 41 Kg/m² with enrichment: 98% ; 41 Kg/m² without enrichment: 100% ; p=0.0001) and 32 days of age (31 Kg/m² with enrichment: 90.7% ; 31 Kg/m² without enrichment: 92.7% ; 41 Kg/m² with enrichment: 98% ; 41 Kg/m² without enrichment: 99.3% ; p<0.0001). Stocking density is often linked to welfare issues like dermatitis (e.g. reviewed in (de Jong et al., 2012), thus this result was expected.

We also noticed a sex effect at 32 days (p=0.02) which was not at 25 days (p=0.36). Males had more severe FPD (score c) than females which had more minor FPD (score b) than males at 32 days of age (25 days: 31.6% of females had severe FPD vs. 35.1% of males and 61.9% of females had minor FPD vs. 58.7% of males ; 32 days: 33.3% of females had severe FPD vs. 42.3% of males and 62.3% of females had minor FPD vs. 52% of males) (Figure 16). It has already been shown that male broilers have higher incidence of FPD than females (e.g.(Shepherd and Fairchild, 2010)), this could notably be related to body weight placed on their footpad, males being heavier than females, exerting more pressure on the litter with the foot pad. Indeed, in our study, males had higher body weights than females.

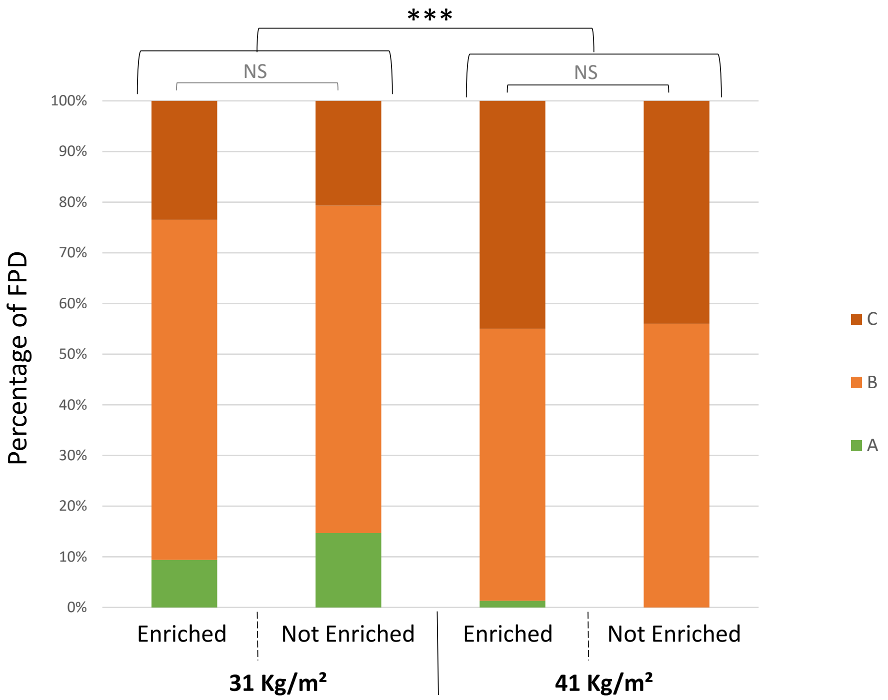

Figure 14: Severity of Footpad Dermatitis on 150 animals by treatment at 25 days of age.
a= no evidence of footpad dermatitis, b= minimal evidence of footpad dermatitis, c=evidence of footpad dermatitis
*NS: Not significant; *** p = 0.0001*

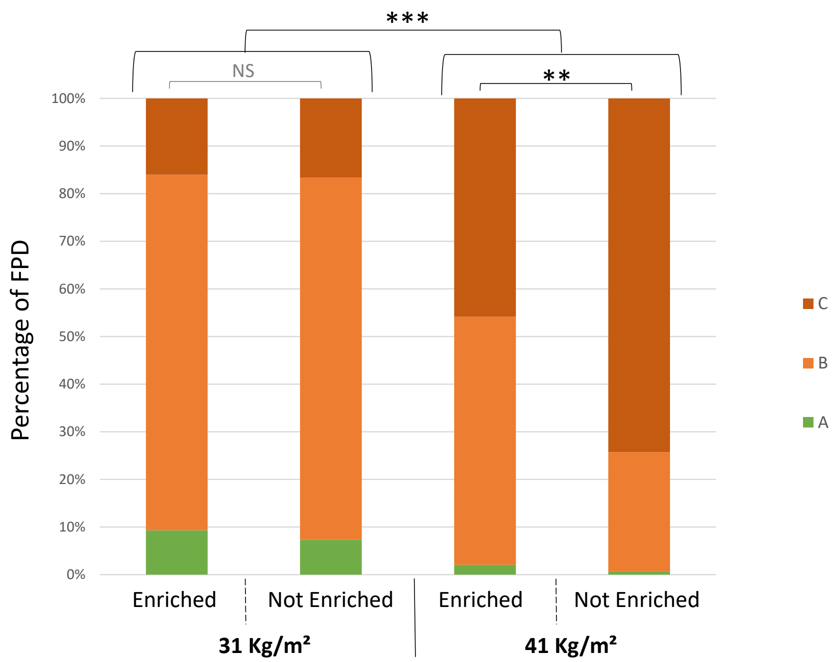

Figure 15: Severity of Footpad Dermatitis on 150 animals by treatment at 32 days of age.
a= no evidence of footpad dermatitis, b= minimal evidence of footpad dermatitis, c=evidence of footpad dermatitis
*NS: Not significant; **: p=0.001; ***<0.0001*

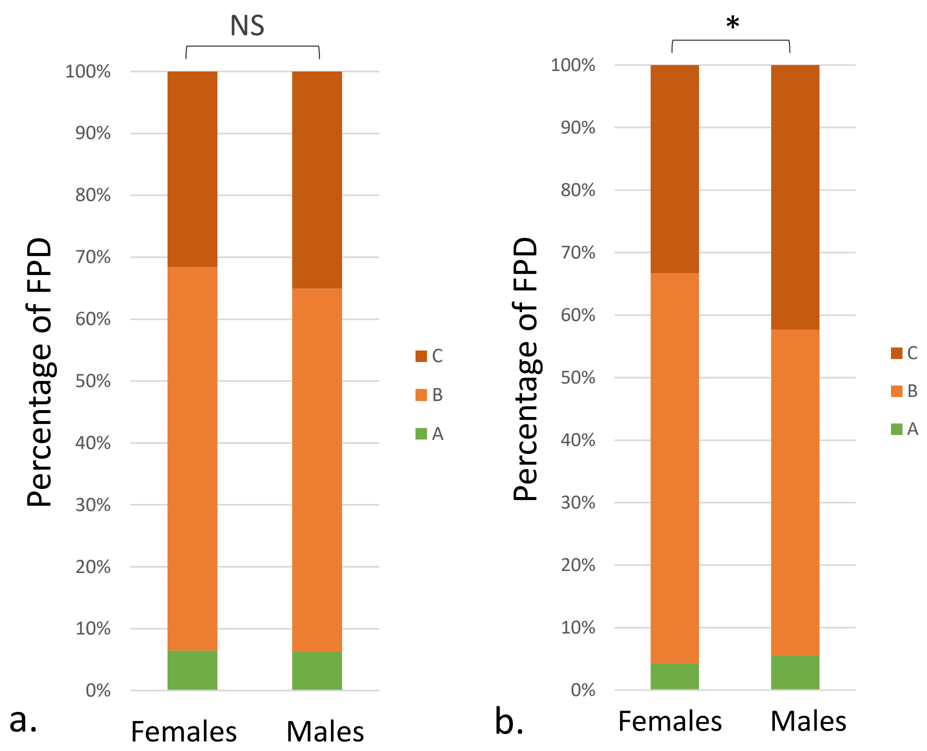

Figure 16: Severity of Footpad Dermatitis on males and females at 25 days (a.) and 32 days of age (b.).
a= no evidence of footpad dermatitis, b= minimal evidence of footpad dermatitis, c=evidence of footpad dermatitis
*NS: Not significant; *: p<0.05*

If we analysed the general results, grouping both stocking densities (25 days and 32 days observations confounded), there was no effect of the enrichment on FPD (p=0.74). These results coincide with previous studies, which did not find enrichment's effect on levels of FPD (Bailie et al., 2018; Yang et al., 2019; de Jong et al., 2021). But we found that at the end of the rearing period (32 days, Figure 15), much more birds reared at 41 Kg/m² without enrichment had more severe FPD (score c) (74.3%) than those raised with enrichment at 41 kg/m² (45.8%) (p=0.001) showing a positive effect of the presence of platforms and straw bales on foot health of broilers housed at a higher stocking density. A previous study of Tahamtani et al. (2020) have shown some potential of platforms to improve footpad health, especially comparing to straw bales (treatments with platforms of 30 cm or 5 cm height with better FPD scores than straw bales' treatment). In our study, we did not differentiate straw bales and platform, which obstructs a potential difference between these two types of enrichment.

In our study, levels of FPD were higher than usual because of some litter management issues. Indeed, in other studies as in Yang et al. (2019) or de Jong et al. (2021), the large majority of birds have a FPD score of 0 (equivalent to our score "a"). However, we can still highlight the positive effect of the enrichment on foot lesions in broilers even with low quality litter conditions.

Hock burns

As for FPD results, we found an effect of stocking density on hock burns. Birds raised at lower stocking density had fewer hock burns than those raised at 41 Kg/m² at 25 days (31 Kg/m² with enrichment: 16.8% ; 31 Kg/m² without enrichment: 14.7% ; 41 Kg/m² with enrichment: 33.5% ; 41 Kg/m² without enrichment: 37.3% ;p=0.0009) and 32 days of age (31 Kg/m² with enrichment: 28.7% ; 31 Kg/m² without enrichment: 22% ; 41 Kg/m² with enrichment: 61.1% ; 41 Kg/m² without enrichment: 66.7% ; p<0.0001) (Figures 17 and 18). Similar to FPD, hock burns are induced by moist and soiled litter, generally impacted by stocking density, but they usually appear later than FPD (de Jong et al., 2012). Same conclusions as above (stocking density effect on FPD) can be drawn. However, unlike FPD, no impact of enrichment was found on occurrence of hock burns during our observations (p=0.62 at 25 days and p=0.17 at 32 days). In previous studies on enriched environment for broilers, similar results on hock burns have been found, but there were only collected data from the slaughterhouse (Bailie and O'Connell, 2014; Kaukonen et al., 2017a; Bailie et al., 2018; Baxter et al., 2020). Only one other study on enrichments in broilers' pens, with hock burns' observations on living animals was found (de Jong et al., 2021) and no effect of enrichments were detected.

In our experiment, males also had more hock burns than females at 25 (females: 20%; males: 31.6% ; p=0.0009) and 32 days of age (females: 33.3% ; males: 55.5% ; p<0.0001) (Figure 19). Hock burns are linked to sex due to the body weight differences between sexes. Males are generally heavier than females, as observed in our study, explaining the higher occurrence of hock burns in males.

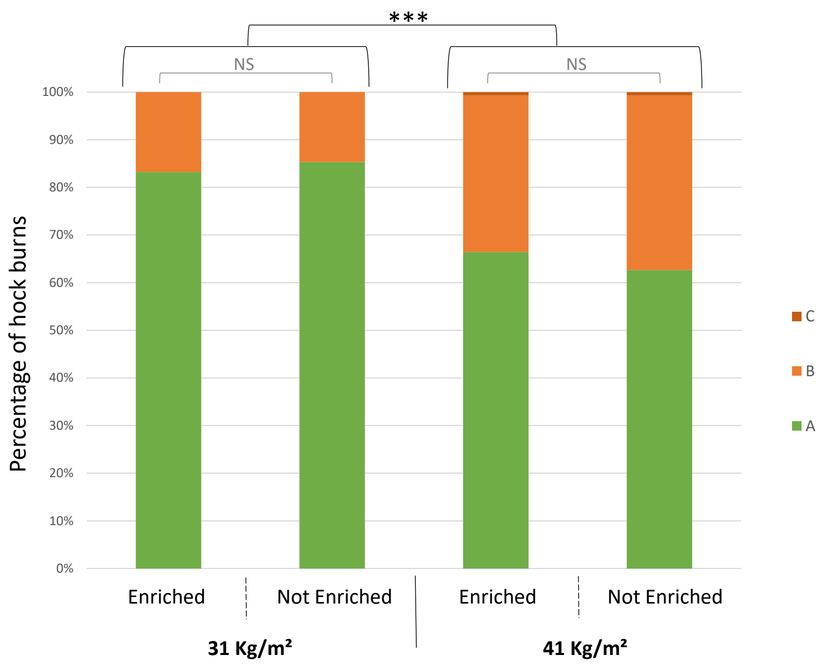

Figure 17: Severity of hock burns on 150 animals by treatment at 25 days of age.
a= no evidence of hock burns, b= minimal evidence of hock burns, c=evidence of hock burns
*NS: Not significant; *** p< 0.0001*

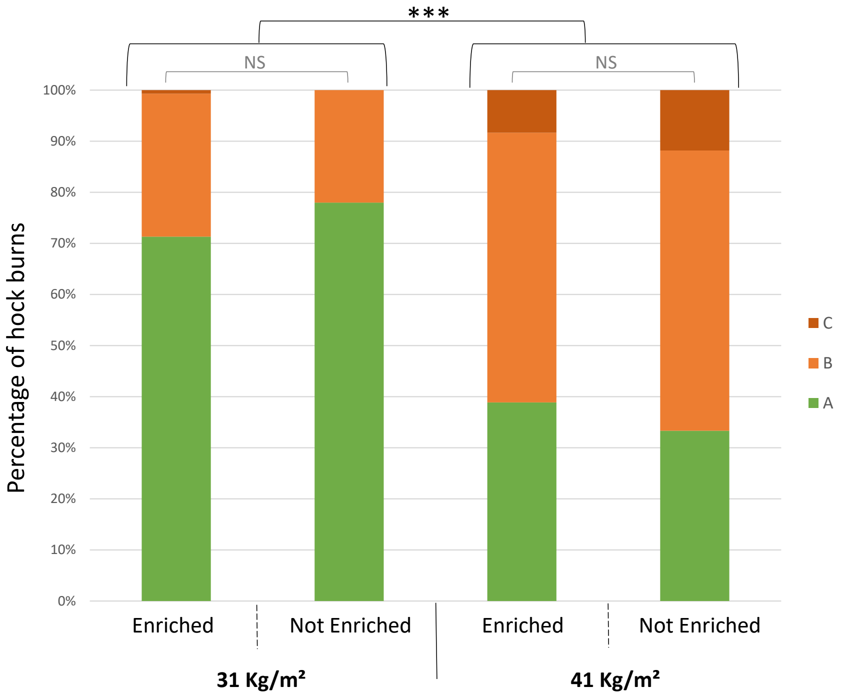

Figure 18: Severity of hock burns on 150 animals by treatment at 32 days of age.
a= no evidence of hock burns, b= minimal evidence of hock burns, c=evidence of hock burns
*NS: Not significant; *** p< 0.0001*

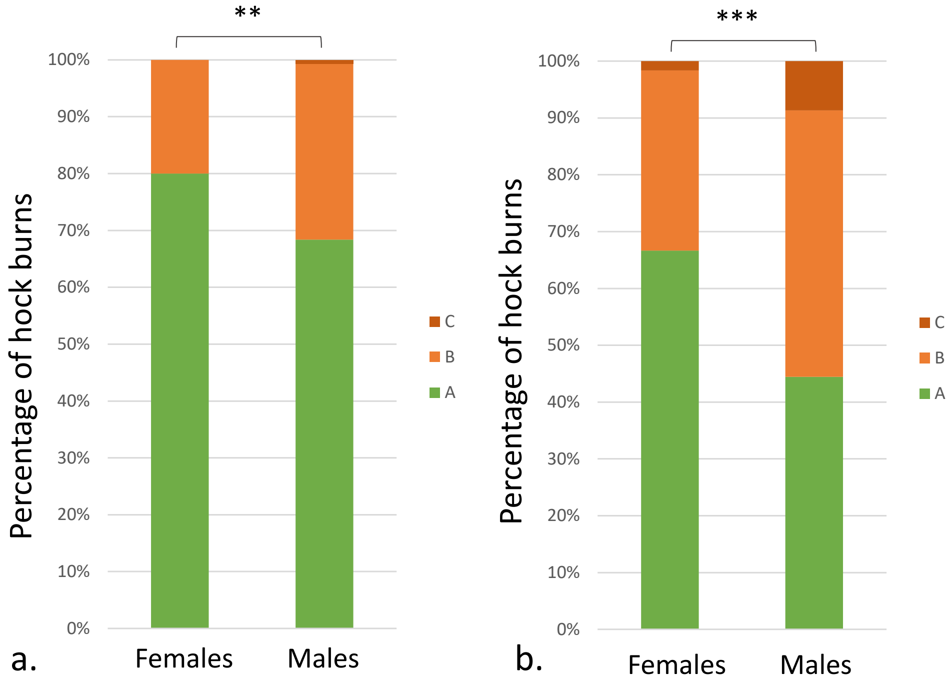

Figure 19: Severity of hock burns on males and females at 25 days (a.) and 32 days of age (b.).
a= no evidence of footpad dermatitis, b= minimal evidence of footpad dermatitis, c=evidence of footpad dermatitis
***: p< 0.01; ***: p<0.0001*

3.7. Post-mortem

<u>Footpad dermatitis</u>

In the slaughterhouse, two major indicators were observed: FPD with a camera system (complete batches analysed) and hock burns by direct observations on the slaughter line on 1850 birds by treatment. As with observation during rearing, we noticed an impact of stocking density on the amount and severity of footpad dermatitis (Figure 20). Broiler chickens raised in pens with the highest stocking density of 41 Kg/m^2 had more severe foot lesions (score c) (31 Kg/m^2 with enrichment: 56.9% ; 31 Kg/m^2 without enrichment: 74.1% ; 41 Kg/m^2 with enrichment: 87.1% ; 41 Kg/m^2 without enrichment: 86.5% ; $p<0.0001$), while more birds raised at a stocking density of 31 Kg/m^2 were observed free of lesions (score a) (31 Kg/m^2 with enrichment: 9.6% ; 31 Kg/m^2 without enrichment: 8.7% ; 41 Kg/m^2 with enrichment: 1.8% ; 41 Kg/m^2 without enrichment: 1.8% ; $p<0.0001$). Otherwise, minor footpad dermatitis (score b) were more numerous in birds raised at 31 Kg/m^2 than at 41 Kg/m^2 (31 Kg/m^2 with enrichment: 33.5% ; 31 Kg/m^2 without enrichment: 22.2% ; 41 Kg/m^2 with enrichment: 11.1% ; 41 Kg/m^2 without enrichment: 11.5% ; $p<0.0001$). These post-mortem results are consistent with our observations on farm concerning the effect of the stocking density. However, we observed an effect of enrichment in broilers housed at 31 Kg/m^2, but not in birds reared at 41 Kg/m^2 while on farm, the opposite was found. Birds reared at 31 Kg/m^2 without enrichment had more severe foot lesions (score c) ($p<0.0001$) and less minor footpad dermatitis (score b) ($p<0.0001$) than those from the 31 Kg/m^2 unenriched group. No difference in FPD scores due to enrichment was observed in birds raised at 41 Kg/m^2 ($p=0.23$) but the levels of FPD in stocking density of 41 Kg/m^2 were critically high, which could explain the absence of enrichment's effect in contrast with the density of 31 Kg/m^2.

In the majority of previous studies, enrichment never impacts FPD levels (Bailie and O'Connell, 2014; Baxter et al., 2017; Kaukonen et al., 2017a; Bailie et al., 2018; Baxter et al., 2020; de Jong et al., 2021) except in Ohara et al. (2015) that found a positive effect of enrichment on females' foot lesions but not on males (females perched more in this study than males), and Tahamtani et al. (2020) who showed a positive effect of platforms on FPD in comparison with straw bales. Nevertheless, in those studies, FPD levels were often very low in contrast with ours (for example, between 80% and 90% of animals had no lesions in Ohara et al. (2015)).

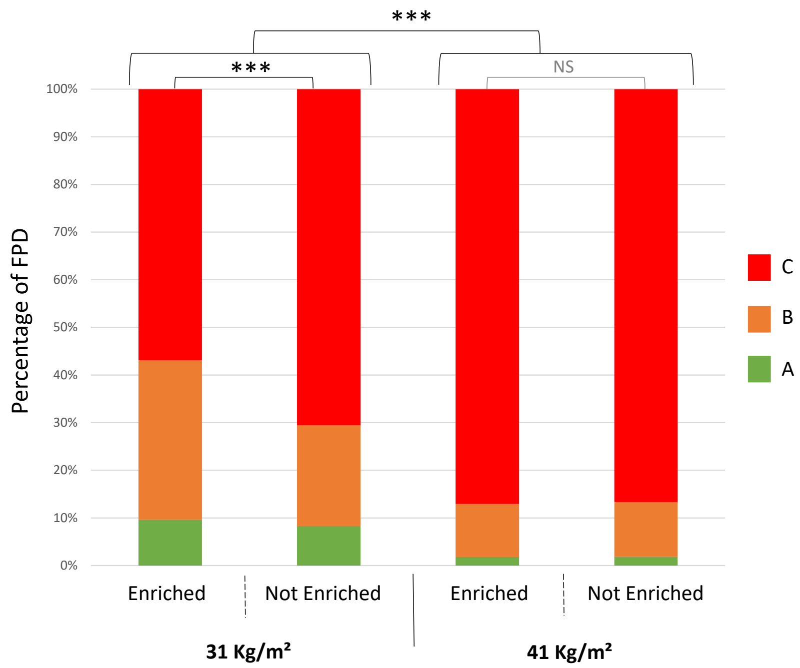

*Figure 20: Footpad Dermatitis of each group in a post-mortem analysis by an automatic camera system at slaughterhouse. ***: p<0.0001*

Hock burns

With the direct observations on the slaughter line, we found that stocking density and enrichment impacted the scoring of hock burns (Figure 21). Broiler chickens raised at the higher stocking density had more severe (score c) (31 Kg/m² with enrichment: 0% ; 31 Kg/m² without enrichment: 0% ; 41 Kg/m² with enrichment: 0.6% ; 41 Kg/m² without enrichment: 3.4% ; p<0.0001) and minor hock burns (score b) (31 Kg/m² with enrichment: 3.6% ; 31 Kg/m² without enrichment: 5.2% ; 41 Kg/m² with enrichment: 18.6% ; 41 Kg/m² without enrichment: 25% ; p<0.0001), whereas birds raised at 31 Kg/m² were more free of hock burns (score a) than the ones reared at 41 kg/m² (31 Kg/m² with enrichment: 96.4% ; 31 Kg/m² without enrichment: 94.8% ; 41 Kg/m² with enrichment: 80.8% ; 41 Kg/m² without enrichment: 71.7% ; p<0.0001). This impact of stocking density on the levels of hock burns at slaughterhouse coincide with our results on farm.

There was also an effect of enrichment in 31 Kg/m² and 41 Kg/m² treatments. Birds from enriched treatment, housed at 31 Kg/m², had fewer minor lesions (score b) (p=0.02) and were more often free of hock burns (score a) (p=0.02) than those raised at the same stocking density without enrichment. At 31 Kg/m², no severe hock burns (score c) was observed. At 41 Kg/m², there were more severe hock burns (score c) (p<0.0001) and minor lesions (score b) (p<0.0001) without enrichment than in enriched treatment. Birds raised with enrichment at the highest stocking density were more often free of hock burns (score a) than birds raised at the same stocking density of 41 Kg/m² without enrichment (p<0.0001). To our knowledge, no previous study has shown an effect of enrichment on levels of hock burns in broiler chickens (Bailie and O'Connell, 2014; Kaukonen et al., 2017a; Bailie et al., 2018; Baxter et al., 2020; de Jong et al., 2021). Thus, our study showed that enrichment may improve hock health.

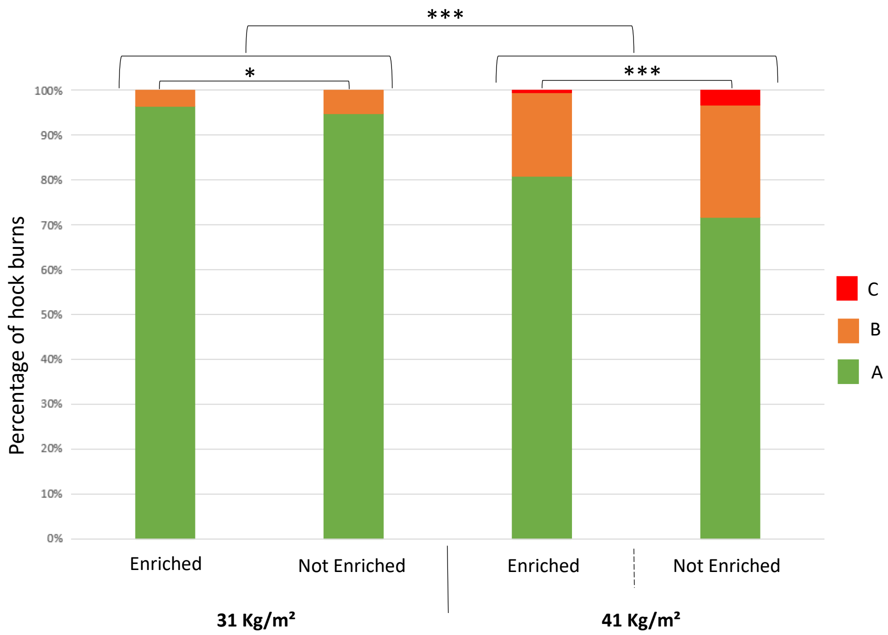

Figure 21: Hock burns of broilers from each treatment at post-mortem.
**: p<0.05; ***: p<0.0001*

Some differences were observed between FPD scoring on farm (Welfare Quality notation scale, visual scoring, on a sample) and at slaughterhouse (automatic detection camera system, on full flock). These variations could be explained by differences in the notation scales and in the sample sizes, and by the disparities between manual and camera scoring. For example, some trials perform in Denmark using camera systems found that there was under-reporting of the incidence of FPD compared to visual assessment although stakeholder industry, in the same country, were claiming the opposite (FCEC, 2017).

4. Conclusion

With our experiment, we wanted to know the impact of enrichments with two different stocking densities for fast growing broiler chickens reared indoor by using Animal Based Indicators and, Resource Based Indicators. Thus, it also allows us to improve our knowledge on some indicators, Animal and Resource Based. Impacts of stocking density and enrichment on animal welfare were found, based on ABI. Besides, indicators like litter quality and gas concentrations were analysed in regard to their validity and/or reliability. Our main results are summarized in Table 6.

Our study succeeds to show that a fast-growing broiler chicken (Ross 308), are using provided enrichments all along the rearing period. Despite their health issues like footpad dermatitis, hock burns, or typical health/behavioural problems for this strain (legs weakness, inactive behaviour,…), they interacted with the platforms and straw bales in different ways (perching, staying under the platforms, clustering around straw bales, pecking straw bales) depending on their age and needs. In addition to the benefit of these enrichments for the expression of natural behaviours, animal welfare was generally improved (walking abilities, footpad dermatitis and hock burns), showing the real and measurable interest of environmental enrichment to fulfil animals needs and improve their welfare.

Nevertheless, our experiment highlights a potential gap of knowledge in the directive's definition of a useable area[4] for space under an enrichment, like in our experimental settings, the plateforms. In our experiment, animals had "access" to this space under the platforms of 30 cm height from their arrival until the end of the rearing period. During the last weeks, they were not able to stand up under the plateforms and remained in a sitting position because of their size (Figure 22). In any case, numerous animals where seen sitting under the platforms, (unfortunately, the lack of visibility did not allow us to systematically count the exact number of animals staying underneath). The main hypothesis explaining this behaviour is that the space under the platform could act as a shelter for the birds, where they can rest undisturbed by other animals. In this situation, the space underneath the platform (of 30 cm height) might be considered as "useable area" by animals. Further investigations should be done to compare the different height of platforms and their occupancy and capacity to fulfil animals needs.

Last but not least, this experiment allowed investigation about ways to measure welfare indicators. This was the case for example of automatic or visual assessment of FPD or litter scoring at the farm. Results allow us for example to suggest that Classyfarm scoring systems seems more valid than welfare quality one but further investigations are needed.

This experiment will lead to the publication of a scientific paper.

Figure 22: Broiler chickens staying above and under a platform ©Anses

[4] « Littered area accessible to the chickens at any time » - Council Directive 2007/43/EC of 28 June 2007 laying down minimum rules for the protection of chickens kept for meat production.

Table 6: Main results summarised

INDICATORS	RESULTS	CONCLUSIONS
Air quality	No enrichment or stocking density effect on NH_3 and CO_2 concentrations. Regarding measurement: No difference of gas concentrations between above and underneath the platforms. No difference of gas concentrations between above or underneath the platforms and the rest of the pens.	Enriching the broilers' environment did not decrease or increase the gas concentrations, regardless of stocking density. The presence of a platform did not imply a modification of gas assessment protocol. There is no need to specifically measure the gas concentrations around platforms. However, our levels of NH3 and CO2 were quite low in our experimental facilities. These measurements need to be repeated in commercial farms situations.
	No enrichment or stocking density effect on dust level.	Enriching the broilers' environment did not decrease or increase the dust level, regardless of stocking density. However, our levels of dust were quite low in our experimental facilities. These measurements need to be repeated in commercial farms situations.
Litter Quality	No enrichment or stocking density effect on the whole pen litter humidity. However, the litter was more humid under the platform than in the rest of the pen.	Enriching the broilers' environment did not impact the litter humidity, regardless of stocking density. However, in case of non-addition of litter underneath the platform, the litter moisture will increase faster because of droppings from animals staying above and under the platforms
	Classyfarm (Vinco et al., 2020) and Welfare Quality (WelfareQuality®, 2009) litter assessment protocols are reliable between two observers. Results from these two protocols were similar (correlation). Classyfarm scoring system was also correlated with litter humidity measurements	The reliability between these two scoring systems needs to be tested with more observers. Because of practical constraints, the litter quality assessments and litter humidity measurements had not been done the same day. This needs further experimentation before any conclusion can be made.
Use of enrichments	<u>Straw bales:</u> - Pecking and clustering around straw bales occur more at early ages. - Birds from the 41 Kg/m^2 pen perched more on	Further research on angles of access ramps needs to be done.

	straw bales than those from the 31 Kg/m² pen <u>Platforms:</u> - Platforms were occupied throughout the rearing period. - Space under the platform was almost all the time completely occupied. Broilers occupied it in a standing position at early ages and later in a sitting position.	
Lameness	Enrichment had a positive effect on walking ability at a stocking density of 41 Kg/m² at 26 days of age. At 26 and 32 days of age, a negative impact of stocking density was found only in non-enriched treatments.	At the end of the rearing period, enrichment appears to mitigate the negative consequences of high stocking density on the walking abilities of broilers. Two hypothesis can be done. Enrichment could affect positively the bone strength of birds and/or limit footpad dermatitis .
Footpad dermatitis and Hock burns on farm and at slaughterhouse	Stocking density had a negative effect on FPD and hock burns measured on farm and at slaughter. At the end of the rearing period, enrichment reduced significantly the occurrence of FPD for broilers: this was visible for birds reared at 41 Kg/m² when the observations were visually done on farm at 32 days of age and, at slaughterhouse for birds reared at 31 Kg/m². Enrichment reduced occurrence of hock burns observed at the slaughterhouse for both stocking densities.	Increased stocking density negatively impacted the contact dermatitis of broilers but enriching the environment seems to reduce the negative consequences of increased stocking density and improve the footpad and hock health at both stocking densities.

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