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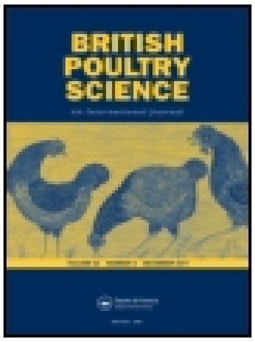
Citation for published version:
Vinco, LJ, Giacomelli, S, Campana, L, Chiari, M, Vitale, N, Lombardi, G, Veldkamp, T & Hocking, PM 2018, 'Identification of a practical and reliable method for the evaluation of litter moisture in turkey production' British Poultry Science, vol. 59, no. 1, pp. 7-12. DOI: 10.1080/00071668.2017.1381334

Digital Object Identifier (DOI):
10.1080/00071668.2017.1381334

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Peer reviewed version

Published In:
British Poultry Science



British Poultry Science

ISSN: 0007-1668 (Print) 1466-1799 (Online) Journal homepage: http://www.tandfonline.com/loi/cbps20

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To cite this article: L. J. Vinco, S. Giacomelli, L. Campana, M. Chiari, N. Vitale, G. Lombardi, T. Veldkamp & P. M. Hocking (2017): Identification of a practical and reliable method for the evaluation of litter moisture in turkey production, British Poultry Science, DOI: 10.1080/00071668.2017.1381334

To link to this article: http://dx.doi.org/10.1080/00071668.2017.1381334

CBPS-2017-113

Ed. Kjaer, August 2017;

Identification of a practical and reliable method for the evaluation of litter moisture in turkey production

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Running Head: Turkey litter moisture assessment

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Accepted for publication 11th August 2017

Abstract. 1. An experiment was conducted to compare 5 different methods for the evaluation of litter moisture.

2. For litter collection and assessment, 55 farms were selected, one shed from each farm was inspected and 9 points were identified within each shed.

3. For each device, used for the evaluation of litter moisture, mean and standard deviation of wetness measures per collection point were assessed.

4. The reliability and overall consistency between the 5 instruments used to measure wetness were high (α =0.72).

5. Measurement of three out of the 9 collection points were sufficient to provide a reliable assessment of litter moisture throughout the shed.

6. Based on the direct correlation between litter moisture and footpad lesions, litter moisture measurement can be used as a resource based on farm animal welfare indicator.

7. Among the 5 methods analysed, visual scoring results the most simple and practical and therefore the best candidate to be used on farm for animal welfare assessment.

Keywords: **f**oot pad dermatitis; welfare; litter moisture; environment; poultry; bedding quality

INTRODUCTION

Wet litter is of major concern to the poultry industry because of its negative impact on animal health, welfare and performance. In particular, foot pad dermatitis (FPD) in turkeys has been shown to be closely related to the content of water in the litter (Mayne *et al.*, 2007) and the prevalence increases linearly with litter moisture above a certain minimum (49% according to

Weber Wyneken *et al.*, 2015; 30% according to Wu and Hocking, 2011). FPD is a contact dermatitis of commercially reared turkeys in which the metatarsal and digital pads become swollen, discoloured and hard (Sinclair *et al.,* 2015). In severe cases FPD may result in ulcerations of the foot pad tissue with detrimental effects on both animal health and welfare. Birds spend most of their productive life in close association with the bedding/litter material and hence the quality of the litter tells a lot about the skin quality of the bird (Abd El-Wahab *et al.*, 2012). For this reason, FPD has been identified as one of the best indicators of animal welfare (AW) in poultry and adopted in the EU legislation on the welfare of chickens kept for meat production.

Although FPD scoring is a reliable welfare indicator as it gives the picture of the conditions in which the animals have been raised, this occurs only after the eventual damage has been done. Thus it would appear more useful to identify a resource based AW indicator that can give indications on how to prevent/reduce the occurrence of FPD, rather than assess these at the end of the production cycle, acting in this way as an anticipated indicator of AW (based on inputs rather than outputs). The monitoring and scoring of litter moisture could therefore represent a valuable resource based animal welfare indicator assisting management, in order to minimise the prevalence and severity of FPD.

Assessing litter moisture content within a turkey shed is not straightforward, as there are wide differences in litter moisture content throughout the barn, along with differences in deep and superficial wetness, the latter likely being more closely related to the occurrence of FPD. Additionally, exposure time of animals to wet litter may vary in field conditions in terms of the time spent around feeding or drinking places, which are particularly "wet litter conditions" (Abd El-Wahab *et al.*, 2012).

Different techniques and guidelines to measure litter moisture have been proposed. In previous studies, litter moisture was determined by drying core litter samples in an oven for different periods of time (from one day to two weeks), measuring the loss in weight of the sample (Abd El-Wahab *et al*., 2012; van der Hoeven-Hangoor *et al*., 2014; Dunlop *et al*., 2016). This kind of assessment is time consuming and requires laboratory equipment. Furthermore, it gives the determination of humidity in core samples, while what really affects the foot pads is the surface of the litter, with which these are in contact.

The purpose of this paper is to identify a practical and reliable method for the evaluation of litter moisture in commercial turkey farms. Five different methods for the evaluation of litter moisture were compared in this trial, carried out within the ERA-NET project TURKEYWELFARE, which started in 2014, with participating research institutes and industries from Italy, UK and The Netherlands.

MATERIALS AND METHODS

The field project started in July 2015 and ended in August 2016. A total of 55 farms, provided by the two main Italian turkey integrations, were selected and one shed from each farm was inspected. The inspection was done towards the end of the production cycle (135-140 days), just before load out.

For litter collection and assessment 9 points were identified, as described below, in order to ensure sampling of the overall litter condition.

For this purpose the right half of each inspected shed was divided into three longitudinal strips (Figure 1): one in the middle of the shed (line A), one near the feeder line (line B) and one near the drinker line (line C). For each line three points were selected: one 6-7 meters from the entrance (I), one in the middle (II) and one 6-7 meters before the end of the shed. (III). Each point is indicated in Figure 1 with a cardinal number.

Figure 1 near here

Litter assessment in each of these 9 points (Figure 1) consisted in the measurement of: (i) deep and superficial wetness with a portable instrument (SM150 Soil Moisture Sensor, Delta-T Devices Ltd, Burwell, UK, which is capable of measuring wetness in a range from 2% to 84,7%, with an accuracy of ± 3%); (ii) total moisture performed in the laboratory, from 300 g samples of whole depth litter using the thermo gravimetric method (electronic wetness analyser Sartorius MA150C, Sartorius mechatronics, Goettingen, Germany) ; and (iii) visual scoring of friability and wetness (Table 1).

Table 1 near here

Measures with portable instruments

The SM150 measures soil moisture content. Its sealed plastic body is attached to two sensing rods which are inserted directly into the soil for taking readings. The soil moisture output signal is a differential analogue DC voltage. This is converted to soil moisture by a data logger or meter using the supplied general soil calibrations. For deep wetness (SM150d) the probe was inserted perpendicularly into the litter (approximately 5 centimetres), while for superficial wetness (SM150s) it was inserted at an angle of 45°, reaching a depth of around 2 centimetres. The calibration for organic soils supplied by the manufacturer of the instrument was adopted to automatically convert the meter readings to litter moisture.

Thermo gravimetric method

The thermo gravimetric method (MP 02/342) was performed at the chemical laboratory of the section of Bologna of the Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (IZSLER). The electronic analyser dries a sample at a temperature of 130° C, until it reaches a constant weight and gives the difference between initial and final weight. This difference determines the amount of wetness lost during the drying and the result is expressed as a percentage.

Visual scoring of friability and wetness

For each point an area of about 1 m^2 was inspected and given a score according to Table 1 (Veldkamp *et al*. 2017, in press). Visual litter quality was determined on a 10 point scale.

Scores for litter friability varied from score 1 = completely caked litter to score 10 = friable litter, no caked litter areas. Scores for litter wetness ranged from score 1 = wet litter (defined as water appearing over the total area when soft pressure was applied by hand or foot to the litter) to score 10 = very dry litter (only observed at start).

Statistical analysis

Exploratory data analysis was performed by boxplots in order to evaluate wetness distribution for each tool (visual friability and wetness scores, MP 02/342, SM150s, SM150d measurements) related to the collection point. Mean and standard deviation were calculated. Since ordinal scales are used in this study, Kruskal-Wallis test was utilised to investigate the relationship between each tool and collection point. In addition, testing the level of associations between tools on different pairs of collection points was investigated through the Goodman and Kruskal's coefficients. Pairwise comparisons using Conover's-test for multiple comparisons of independent samples were used to test the difference between wetness values and position. The Spearman's rho (r_s) statistic was calculated to evaluate the relationship between tools and Fisher's Z transformation was used to calculate an asymptotic confidence interval for $r_{s.}$ The reliability between tools was evaluated by Cronbach coefficient alpha.

Regression analysis was performed on litter score values to estimate values of moisture.

All analyses were performed using R software (R Development Core Team).

RESULTS

Study design: assessing the effect of point collection for each wetness tool.

For each device mean and standard deviation of wetness measures per collection point are summarised in Table 2. MP 02/342, SM150s and SM150d showed lower moisture values in collection points 1, 4, 7 along the middle of the shed (line A) compared to points 2, 5, 8 near the feeder line (line B). Collection points 3, 6, 9 near the drinker line (line C) showed the highest wetness values. The two visual scorings showed a higher value in the points along line A than line B and C. The difference was statistically significant for each tool as the *P*-value associated at Kruskal-Wallis chi square test resulted in $P < 0.0001$ (Figure 2). Friability distribution values were statistically different between lines A and B ($P < 0.001$), A and C ($P < 0.0001$) and B and C ($P < 0.01$).

Figure 2 and Table 2 near here

No difference was observed between the points of line I, II, III (Figure 3) for MP 02/342 (Kruskal-Wallis chi-squared = 1.4602, df = 2, $P = 0.482$), for the two SM150 (Kruskal-Wallis chi-squared = 2.043, df = 2, $P = 0.360$). Also, the two visual scores showed no difference between points of line I to III (for wetness Kruskal-Wallis chi-squared = 1.182, df = 2, $P = 0.554$ and Kruskal-Wallis chi-squared = 0.0876, df = 2, $P = 0.957$ for friability).

Assessing the correlation between wetness tools

Figures 3, 4 and 5 near here

Figure 5 shows the Spearman raw correlation coefficient between the 5 different types of measurements. The highest rank correlation was observed between deep and superficial SM150. The value of 0.93 indicates a statistically significant very strong monotonically increasing relation; as the value of SM150d increased the SM150s value never decreased. The relation between friability and wetness visual scores was also very high (r_s=0.91); the value indicated a very strong statistically significant monotonically increasing relation. MP 02/342 showed a strong monotonically increasing relation statistically significant with SM150d (r_s =0.67) and SM150s (r_s = 0.64). The relation between MP 02/342 and the two visual scores resulted in a moderate monotonically association (friability r_s=-0.45, wetness r_s=-0.48), that was statistically significant. SM150s showed a strong monotonically decreasing statistically significant relationship with the two visual scores. The values of r_s-0.68 with friability and r_s=-0.67 with wetness showed that as the value of SM150s increased the values of friability and wetness tend to decrease. A similar pattern was observed for SM150d and friability (r_s-0.65) and wetness (r_s-0.68).

Values of moisture related to litter scores are shown on Table 3; based on data analysis a wetness visual score of 1 represents an average of moisture >67%, while a score of 10 represents an average of moisture <25%.

Table 3 near here

Assessing the reliability between wetness tools

Reliability between the 5 wetness measures, calculated by the Cronbach coefficient Alpha (α) at a global value, resulted 0.72. As α can be viewed as the expected correlation of two or more tests that measure the same construct, the average correlation of a set of items is an accurate estimate of the average correlation of all items that pertain to a certain construct. As a rule of thumb, a value of 0.70 or higher is considered a good index of reliability between different tools that measure the same trait. Therefore, the value obtained in the study showed good internal consistency related to wetness for the 5 tools used. For the two visual scores α was 0.94, while for SM150s and SM150d α was estimated to be 0.97. These values confirmed good reliability between the two tools and also the possibility to use one tool as a proxy of the other.

Between MP 02/342 and SM150s α was 0.83: This value suggests a moderate reliability between MP 02/342 and SM150s and also the possibility to use one tool as a proxy of the other whereas the α between MP 02/342 and SM150d was lower (α=0.73). The Cronbach coefficient α between MP 02/342 and visual score (friability wetness) resulted in a very low value of 0.45 and 0.47 respectively.

The reliability of SM150s and SM150d for each measurement estimated by α was 0.91.This value represents a good reliability. While the reliability of MP 02/342 for each measurement estimated by α was the lowest observed (α 0.76); as for friability it was 0.87 and for wetness 0.86.

DISCUSSION

As expected, litter moisture showed higher values under the drinker and the feeder lines (lines C and B) and the differences were statistically significant for each tool. Birds tend to crowd in this area to reach feeders and drinkers and furthermore it has been reported that poultry tend to defecate while drinking (Taylor *et al*., 2001). Unavoidable water spillage is another reason. This is exacerbated in the case of inadequate drinker management with unavoidable spreading of the moisture to the surrounding area, affecting litter quality of line B. Line A is in the central area of the shed, which the birds use as a resting area as they are less disturbed by being far away from drinkers and feeders. This tends to compromise the litter in these areas. In the present study, no statistical differences were observed between the points of line I, II, III for all the 5 tools, so it is sufficient to evaluate litter quality in only one of these points with time saving and less disturbance to the animals: the operator can stop at the beginning of the shed, 6-7 meters from the entrance, inspect litter moisture in the middle of the barn, under the feeder and drinker line and have a reliable idea of the overall litter condition in the shed. Visual scores show a very strong correlation with SM150s and SM150d, but not with MP 02/342, probably due to different targets of the two methods: MP 02/342 measures water content of litter, whereas visual scores determine the effect that moisture has on litter appearance.

In this study, the reliability and overall consistency between the 5 instruments used to measure wetness were high (α =0.72). This means that the visual scores (friability and wetness), SM150 (superficial and deep) and MP 02/342 are measures of the same construct. α values increase when the correlations are maximised because the tools are related. Therefore, the possibility of using one tool as a proxy of the other permits one to choose the most suitable method, based on operator's needs, and to compare results obtained with different tools.

In particular, the very strong correlation demonstrated between the two visual scores and those of the two instrumental methods (SM150s and SM150d) is an important outcome in terms of on farm application. The fact that visual scoring, although being a subjective method of litter assessment, can adequately replace the use of more complex instrumental methods, suggests that it as a good candidate to be used on farm for animal welfare assessment. Based on the results of the current investigation, scoring of litter on farm can be done in a very simple, practical and reliable way. Finally, considering the demonstrated direct correlation between litter moisture and FPD, visual scoring can replace the need to perform on farm scoring of footpads. The assessor can simply take an assessment of the litter 6-7 meters from the entrance of the shed focussing his attention on the 3 indicated points, fill out the checklist with no need to introduce instruments or wander about the shed. Visual scoring of litter in this way seems to be reliable, easy to conduct, time saving and with little or no disturbance to the birds.

ACKNOWLEDGEMENTS

The authors acknowledge the cooperation with industrial partners: Aviagen Turkeys, Chester UK; Unaitalia (Italian Meat and Egg Association), Rome, Italy. Società Agricola La Pellegrina (Gruppo Veronesi), Verona Italy. GESCO Società Cooperativa Agricola (Gruppo Amadori), Cesena Italy. No potential conflict of interest was reported by the authors. The experiment was funded by the FP7 ERA-Net ANIHWA, "Coordination of European Research on Animal Health and Welfare" project TURKEYWELFARE [number 182]. The Roslin Institute is supported by an Institute Core Strategic Grant from the Biotechnology and Biological Sciences Research Council (BB/J004316/1).

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FIGURE LEGENDS

Figure 1. Collection point distribution in the shed. The right half of each inspected shed was divided into three longitudinal stripes (Figure 1): one in the middle of the shed (line A), one near the feeder line (line B) and one near the drinker line (line C). For each line three points were selected: one 6-7 meters from the entrance (I), one in the middle (II) and one 6-7 meters before the end of the shed. (III). Each point is indicated in Figure 1 with a cardinal number.

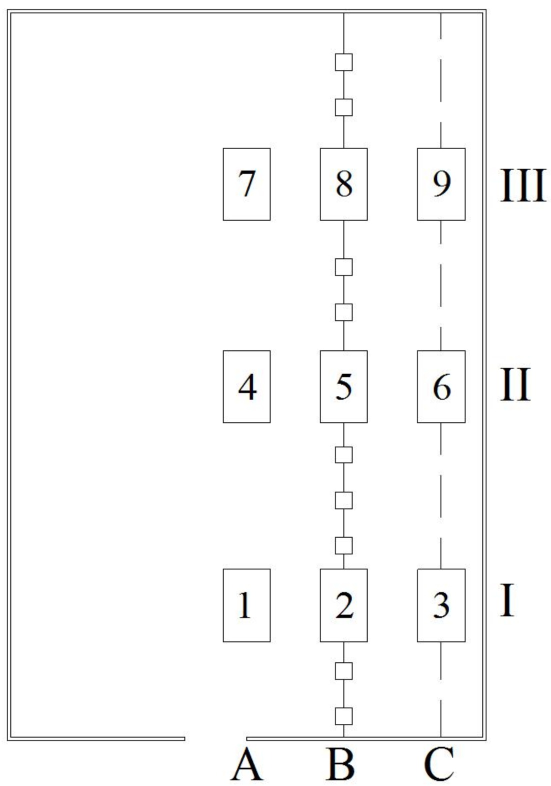

Figure 2. Boxplot of wetness value distribution SM150d, SM150s, friability and wetness by lines. Line A was in the middle of the shed, line B was near the feeder and liner C was near the drinker line. Median values measured by each method were statistically significant as showed by Kruskal Wallis *P*-value included in the plot.

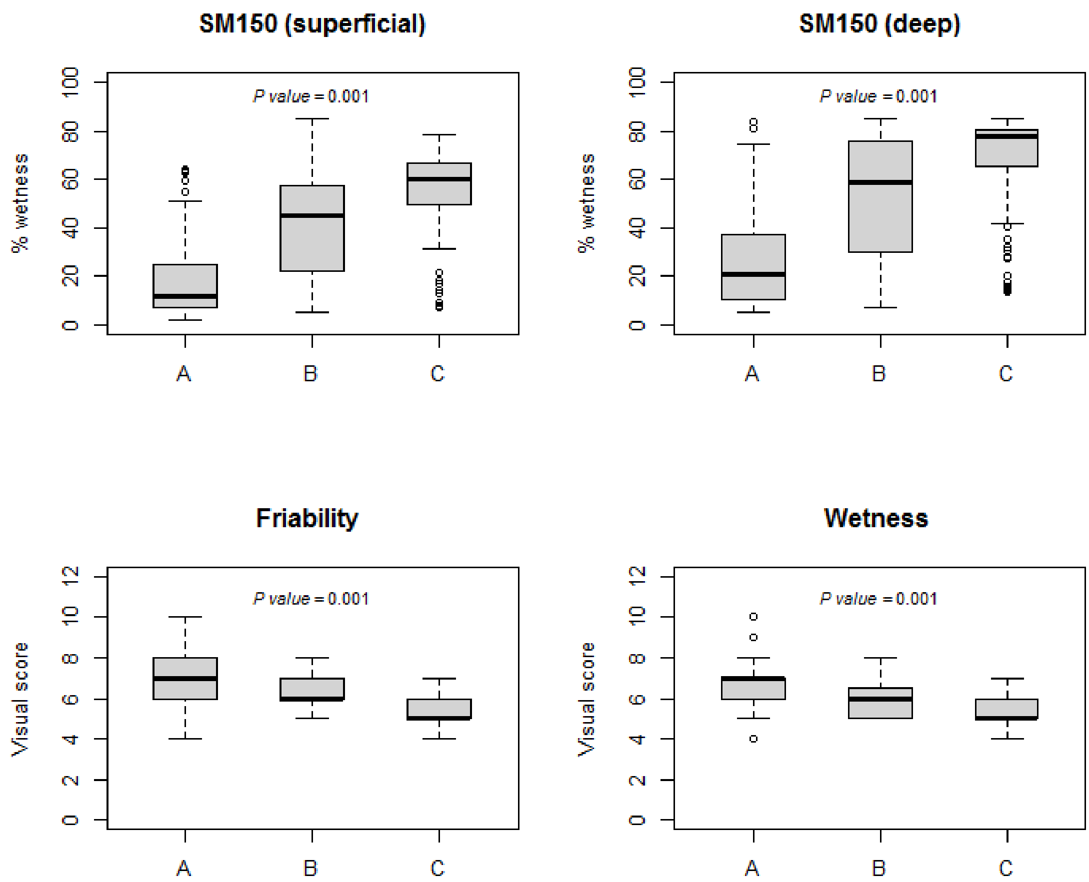

Figure 3. Boxplot of wetness value distribution SM150d, SM150s, friability and wetness by point. Point I was 6-7 meters from the entrance, point II was in the middle and point III was 6-7 meters before the end of the shed. Median values measured by each method were not statistically significant as showed by Kruskal Wallis *P*-value included in the plot.

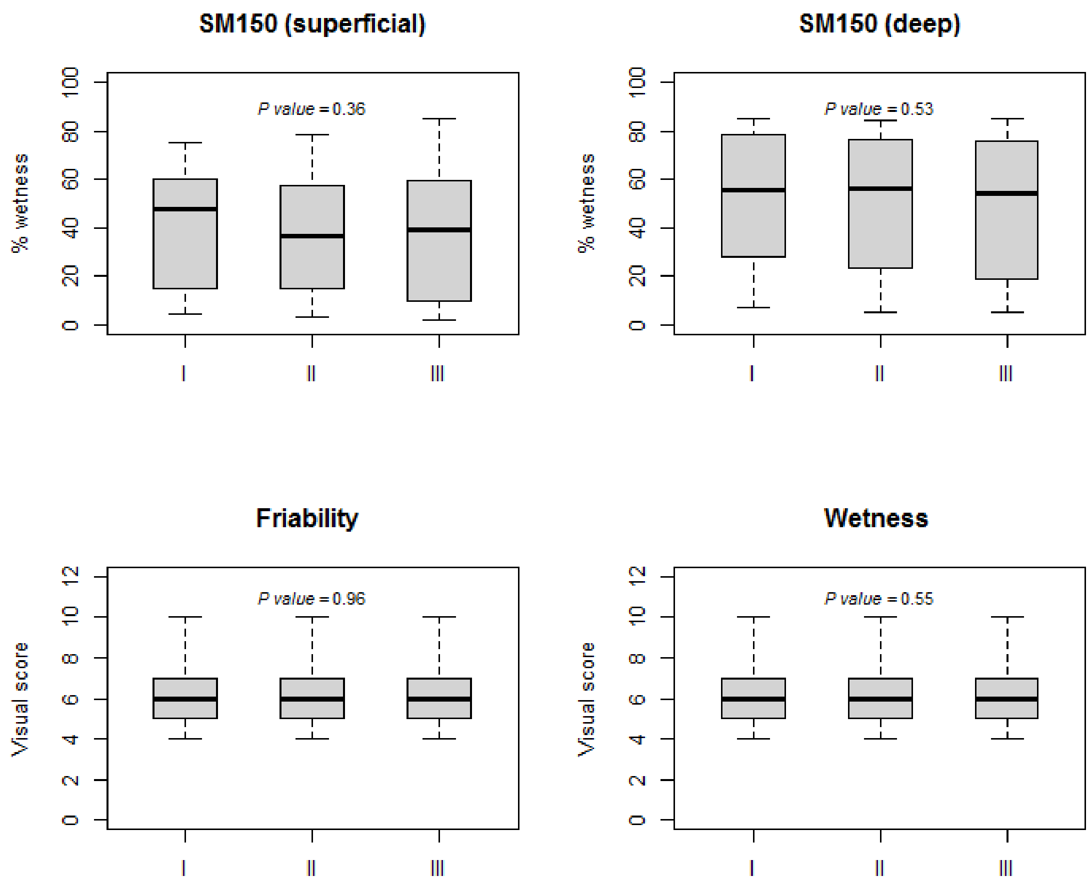

Figure 4. Boxplot of wetness value distribution of MP 02/342 by points and lines. Kruskal Wallis *P*-value are included in the plot.

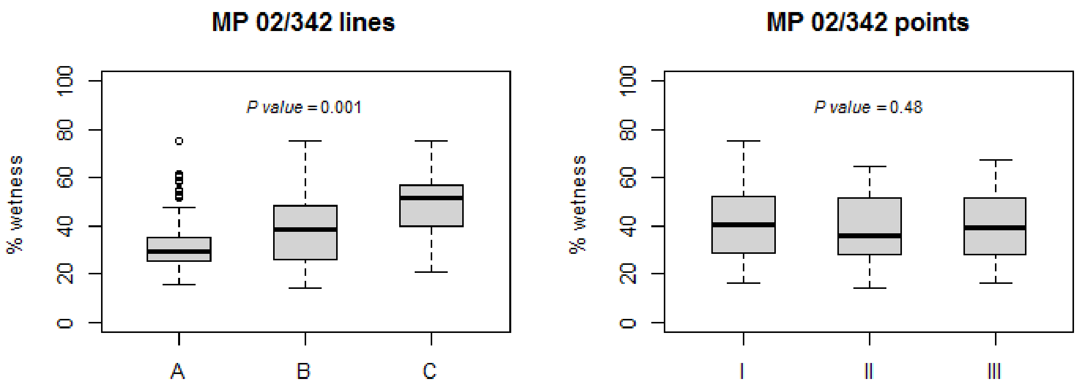

Figure. 5. Correlation matrix and pair plots of all 5 wetness tools measurements are displayed. Each dot plot displays the rank values of the horizontally and vertically projected pair of wetness device indicated in the diagonal. Spearman rank correlation coefficients with significance levels of a two-tailed t-test associated to Spearman rank coefficients are given above the description in the diagonal panel (*** *P*-value < 0.001).

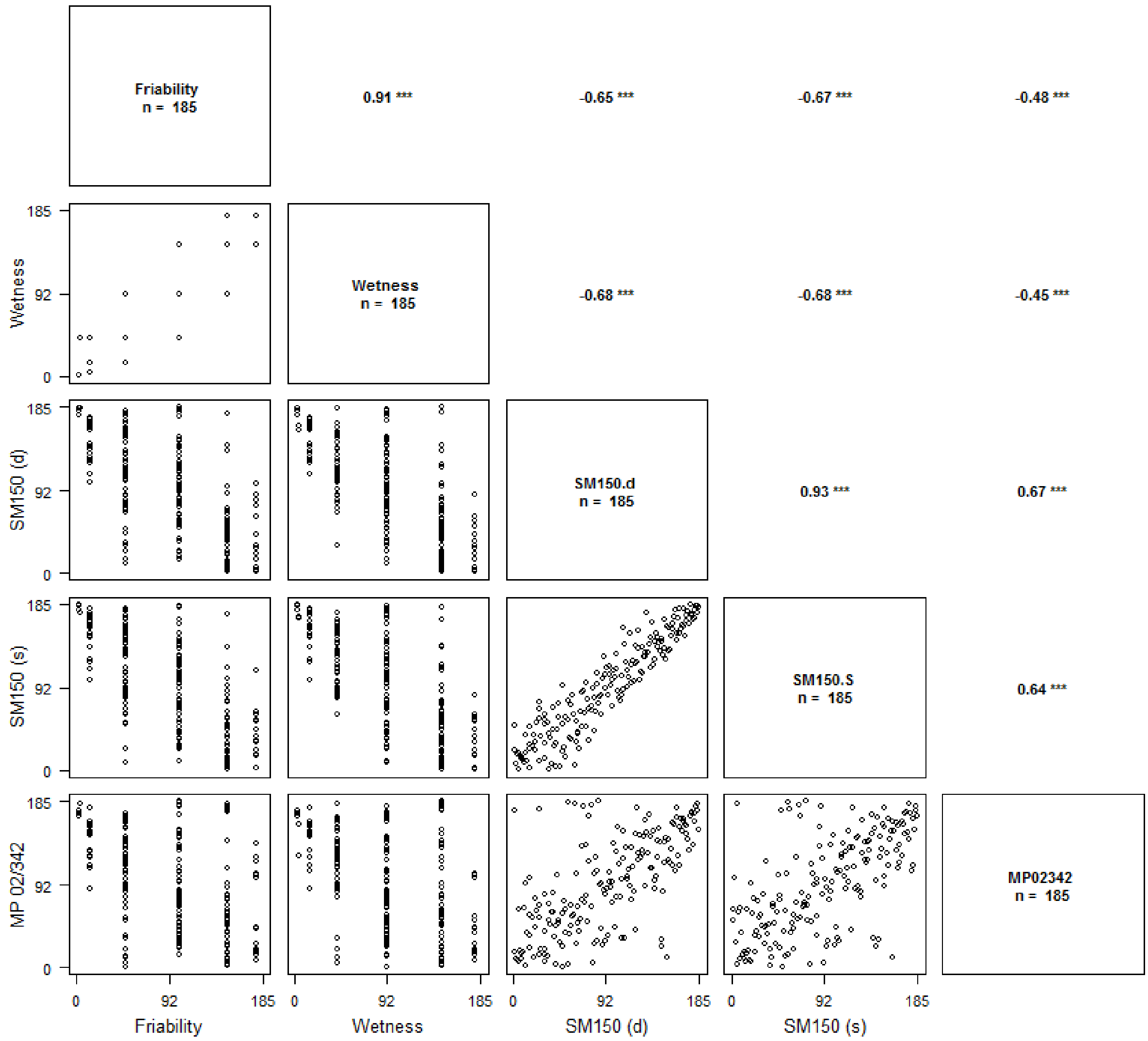

Table 1. *Description of the visual litter scores for friability and wetness*

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)

Just started ridges: slightly visible (see Supplemental Figure for photograph).

Small ridges: well visible beneath drinking line.

Larger ridges: well visible, overmatching the drinker rim.

Supplemental Figure referred to here to be in electronic version only

Table 2. *Mean value and standard deviation (±) for each collection point in the shed, data from visual scoring and wetness measures devices (n=55 sheds)*

Collection point	Visual score of friability	Visual score of wetness
1	6.67±2.8	6.39±2.85
2	6.05±1.9	5.84±2.24
3	5.32±3.5	5.11±3.64
4	7.06±2.4	7.06±2.33
5	6.11±2.2	5.94±2.47
6	5.33±3.4	5.22±3.68
7	7.17±2.4	7.33±2.21
8	6.22±2.5	6.17±2.59
9	5.33±3.4	5.22±2.69

Table 3. *Moisture values, expressed in %, were estimated by regression analysis on wetness visual scores*

Wetness visual score values	% Moisture
Score 1	≥67%
Score 2	66-62%
Score 3	61-57%
Score 4	56-52%
Score 5	51-45%
Score 6	46-41%
Score 7	40-36%
Score 8	35-31%
Score 9	30-26%
Score 10	≤25%

SUPPLEMENTAL MATERIAL LEGEND

Supplemental Figures

Figure A: Just started ridges: slightly visible

Figure B: Small ridges: well visible beneath drinkers.

Figure C: Larger ridges: well visible, overmatching the drinker rim

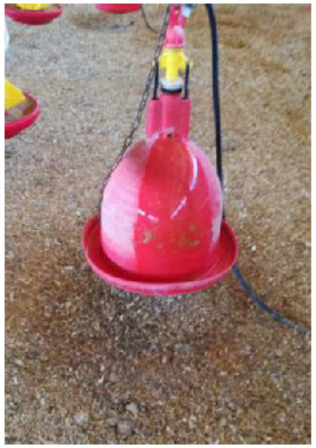

Figure A: just started

Figure B: small ridges

Figure C: larger ridges

Figure A: Just started ridges: slightly visible

Figure B: Small ridges: well visible beneath drinkers.

Figure C: Larger ridges: well visible, overmatching the drinker rim