Living Organisms And Biodegration Changes Of Poultry Litter During Breeding And Their Relation To Chicken Health And Poultry Products Safety

Soares CE, Dahlke F, Maiorka A, Moecke EHS, Dutra MO, Scussel VM

***Abstract*— Contamination and biodegradation changes of poultry litter that may occur during the breeding stages of poultry and may affect chicken health and meat product safety have been studied. The living organisms most isolated period were insects (mainly Alphitobius sp. and Liposcelis sp.), mites (Acarus sp. and Dermanyssus sp.) and fungi (Trichoderma sp., Aspergillus sp., Fusarium sp. and Penicillium sp.). The poultry litter biodegradation that occurred throughout the breeding period, lead to (a) an increase on its pH (6.3 to 8.7) and humidity (mc: 9.7 to 40.6%; - aw: 0.74 to 0.98); (b) change on the texture characteristics, color and reduction on particles size (regular to as small as<10 mm i.e., 34 to 88% of total). In scanning electron microscopy, were registered as cell wall disintegration and tissue fungi infections. Changes detected, can lead to reduction of animals speed and possible fungi toxins contamination.**

***Index Terms*— chicken, fungi, insects, meat, pine shavings, poultry litter, residue**

I. INTRODUCTION

Poultry litters are utilized for poultry(chicken/turkey/ducks) accommodation during their development and are composed initially of only dry cellulosic materials (which may vary regarding origin and dimensions). They can be made from sliced/ fine wood fragments (shavings/sawdust), grain husks (rice/wheat) or just grass providing birds thermal comfort and environment humidity absorption. They protect animals from soil direct contact and control shed's temperature fluctuation. The Poultry litter should be free of foreign matter and living organisms (insects, fungi, bacteria). It is also called poultry/avian bed or just poultry litter. By the time of its discard (at the end of the 45 days period) or re-utilization as fertilizer (Fernandes, 2004; Williams, 2013; Bolan et al., 2010).

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Chicken feed residues (ground corn) and animal wastes (faeces with urea) together with high humidity (especially near the drinkers & feeders) make Poultry litter an optimal environment for living organisms growth, which interferes to poultry development, health and well-being (Nadia et al., 2015). In addition, the internal environment conditions, when reaching also high relative humidity / rainy days and temperature, are optimal for fungi (both deteriorating and toxigenic). Apart from foreigner matters presence and environment favorable conditions, the Poultry litter matter itself (i.e., the cellulosic material utilized), becomes attractive to several deteriorating living organisms attack, that is responsible for its biodegradation (Cavalcante. 1982; Nadia et al 2015).

The insects that infest Poultry litters reported in the literature that are considered the main poultry farming pest worldwide are beetles from the *Alphitobius* genus, especially the *A. diaperinus* Panzer1797. Their presence (larvae stage) is detected in the poultry sheds compacted soil and can reach (be ridden) 80 cm depth (Chernaki-Leffer et al., 2002). They concentrate mainly close to the drinkers and feeders, also inside the shed structures wooden materials. In a study carried out by Skov et al. (2004) and Soares et al. (2018), authors identified different beetle species (*A. diaperinus, Typhaea stercorea*, *Ahasverus advena* and *Carcinops pumilio*), being the first detected in all samples surveyed. Other insects such as *Liposcelis* sp. and *Musca domestica* L.) can be also present (Nayak et al., 2014). Those insets are important poultry safety wise because, apart from interfering to chicken well-being, they are other living organisms (mites, fungi and bacteria) carriers (Lambkin et al., 2007; Banjo and Adeduji, 2005).

Mites (*Dermanyssus* sp.) are reported being highly detected and are considered also one of the main poultry production pests. Apart from causing allergies, the hematophagous mites (such as *Dermanyssus* sp. and *Ornithonyssus* sp.) only parasite the chicken during their blood meals activities and keep the rest of their life hidden in the shed. Some of them are susceptible to fungi (*Trichoderma album* and *Beauveria baussiana*) and others utilize them as their main food source (Kaoud, 2010; de Souza, 2014). In addition, because sheds are attractive to rodents such as mice (*Mus musculus* L.) due to food, water and shelter opportunities, they become also mite potential carriers (Chauve, 1998;Mul and Koenraadt, 2009).

Regarding fungi, they biodegrade cellulosic material and can be identified as *white, brown* and *soft -rot* fungi, that are able to degrade the plant cell wall three components (cellulose, hemicellulose and lignin) (Lazarotto et al., 2016). Fungi poultry litter degradation begins with the hyphae

penetration through the wood cell lumen and by secreting enzymes, catalize the plant cell wall components breakdown (Kirk, 1998). Some fungi such as *Fusarium* sp., *Trichoderma* sp. and *Rhizopus* sp. are responsible for more resistant cell wall components degradation (cellulose and hemicellulose) and *Fusarium* can be toxigenic, being another problem to poultry breeding - the toxins formation (Scussel et al., 2014; Savi et al., 2016).

Considering that poultry litters (a) stay in contact with the animals during the whole breeding period and (b) are exposed to high humidity and foreigner matters (feed residues / animal wastes): this work evaluated the poultry litter (from dry pine – *Pinus taeda* L. - shavings) safety, regarding living organisms contamination, its biodegradation (that occur during chicken breeding) changes, and its relation to animals health, well-being extensive to meat products.

II. MATERIALS AND METHODS

1.1 Material

(a) Sample: (a.1) avian bed (2 kg), made from pine wood fragments (shavings) utilized during the whole breeding stage (after birds housing - Day 3, 21 and 45) and only *(a.2) pine fragments* (1 kg) as *Control* (prior housing) (Day zero).

(b) Culture media and reagents: potato dextrose agar (PDA) and peptone bacteriology media were from Himedia (Curitiba, Parana, Brazil) and chloramphenicol from Vetec (Duque de Caxias, RJ, Brazil), phenolphthalein and sodium hydroxide, from Merck (Darmstadt, Germany).

(c) Equipment: autoclave, Phoenix (Araraquara, SP, Brazil); microwave oven, Philco (Sao Paulo, SP, Brazil); tweezers, Prolab (São Paulo, SP, Brazil); caliper, DigimaticR (Mitutoyo, Union King); drying oven, Olidef-cz (Ribeirao Preto, SP, Brazil); a_w meter, Aqua- Lab4TE, Decagon (Sao Jose dos Campos, SP, Brazil), pHmeter, Schott-gerate CG818, Schott (Mainz, Germany); laminar flow cabinet, Veco (Campinas, SP, Brazil); fume cabinet, Quimis (Diadema, SP, Brazil); rotary shaker, Marconi (Piracicaba, SP, Brazil); microbiological incubator, Quimis (Diadema, SP, Brazil); colonies counter, Phoenix (Araraquara, SP, Brazil); sieve system, (mesh: 2-1mm), Beffer (Caieiras, SP, Brazil). Microscopes - light (LM),(400x) CH-Bl45-2, Olympus (Shinjuku, Tokyo, Japan); stereo (SM), (180x) model Opzt, coupled to a color image-capture camera, model OPT14 MP, Opticam (Doral, Fl., USA) ; and scanning electron (SEM), (5000x), JSM-6390LV, Jeol (Peabody, Mass, USA).

(d) Experiment site: (d.1) poultry shed - capacity for 15.000 birds, dimensions of 100 x 12 x 3 m for length, width and height, respectively (total area: 1200 m^2); polyethylene curtains; concrete floor, hexagonal nets (2.5 cm mesh) and polypropylene ceiling; *(d.2) poultry litter* (pine shavings) - spread on the 240 m^2 (1200 m^2 x 0.2 m), layer being 20 cm thick. No pesticide (neither insecticide nor fungicide) applied, located at latitude 27º 86' and altitude 48º 94'. Total weight prior chick housing 20.400 kg.

1.2 Methods

(a) Sample collection and preparation: (a.1) poultry litter collection - samples (500 g portions) were collected representatively from the shed's floor (10 cm layer deep) after the birds housing (at Day 3, 21 and 45); *points of collection*:4 points (n= 5/each point) of the total shed´s area (Figure 1). The total area utilized (m^2) / growth stages:12x30, 12x60 and 12x100 m^2 for stages A (up to 3 days), B (from Day 4-21th) and C (from 22- 45th), respectively. *Control*: prior birds housing, pine fragments samples (100 g/point) were also collected. Samples types, were stored in sterile polyethylene bags at 8ºC and sent to the Laboratory of Mycotoxicology and Food Contaminants of CAL/UFSC; *(a.2) sample preparation* - each sample was homogenized and divided into 2 different portions (ground and whole) to carry out the investigation of living organisms contaminations and biodegradation changes, respectively. Note: for SEM analysis, the poultry litter fragments were cut into cubes and prepared as reported by (Scussel et al., 2014b; Kreibich et al (2017) i.e., fixed on stubs containing double sided carbon tape, and gold coated, under vacuum.

(b) Living organisms contamination:(b.1) insects, mites & others- they were collected / separated from the poultry litter (50 g portions) samples (whole/live/dead/fragments– including larvae and pupae) by sieving (2-1mm) and utilizing tweezers (and *Control*), then had their characteristics identified by LM, SM and SEM (with different amplifications), percentage calculated (per animal growth stage) and then correlated to the *Control* (Soares et al., 2018); *(b.2) total fungi load* - the enumeration technique was applied (Silva et al., 2010). Briefly, each sample (25 g) was added to peptone solution (0.1%), stirred on a rotary shaker (2 min), then dilutions (10^{-1}, 10^{-2}, 10^{-3}) were spread (0.1 ml) on PDA (n=2) surface (with chloramphenicol) and incubated (7 days, at 28°C in the dark). The results were reported as colony forming units per gram (CFU g^{-1}) in the dilution 10^{-1}. All experiments were carried out at Day zero (*Control*) and after, 3, 21 and 45 days of birds housing. Fungi colonies were also identified/visualized by SEM (as in 2.2.a.2).

(c) Poultry litter biodegradation changes: both, the poultry litter (chicks post-housing) changes and the pine fragments (*Control*) characteristics were investigated throughout the periods as follows *(c.1) physicochemical analysis* - pH, acidity and mc were determined by the international official AOAC methods (AOAC, 2005) and the a_w was through the Aqualab apparatus at 25ºC (n=3) (Decagon, 2001);*(c.2) macro &microscopic observation* - (*c.2.1) macroscopy*-the characteristics of *texture, color* and *deterioration/stains* changes of particulates were registered by SM; their *dimensions (mm)*i.e., *length* (mm) was obtained by utilizing caliper; *weight (g)* by sieved particulates size separation - smaller than 10 mm mesh (<10 mm) & equal to/or higher than 10 mm mesh(□10 mm), then weighed (analytical scale) and percentage (%) calculated to check their decomposition intensity degree. The *foreign matter/impurities*(feed residue/animal wastes/loose feathers/others) were separated and identified by SM; also by*(c.2.2) microscopy* - performed using different microscopes (LM / SM / SEM) for their tissues changes and so fungi and insects details at different magnifications.

Statistics: the software statistic 13 was utilized for the analysis of dimensions, mc, pH and their variation expressed as average ± SD.

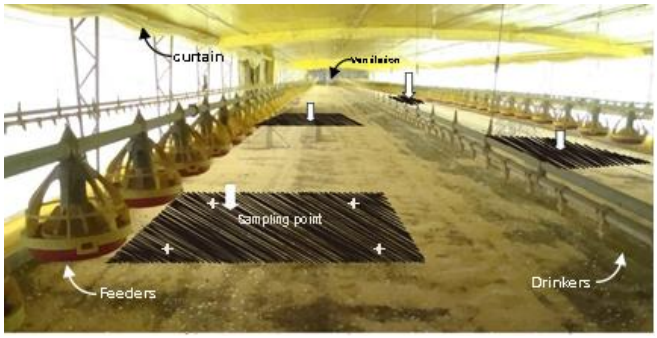


Figure 1. Poultry shed poultry litter sample collection points(+) during the birds 45 days growth period.

III. RESULTS AND DISCUSSION

From the data obtained on the poultry litter living organisms (insect/mites/fungi contamination) and biodegradation (physicochemical and macro/microscopic changes),it was possible to detect several alterations that can interfere on the animals well-being and health. Table 1 and Figures 2-5 show the poultry litter contamination levels and biodegradation changes over the chicken breeding 45-days period, including their effect on animals and poultry products safety.

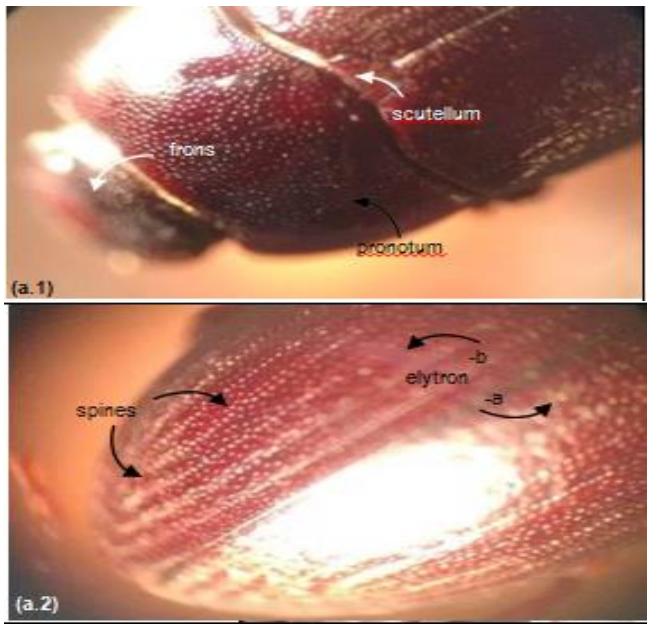
A. Living organisms contamination

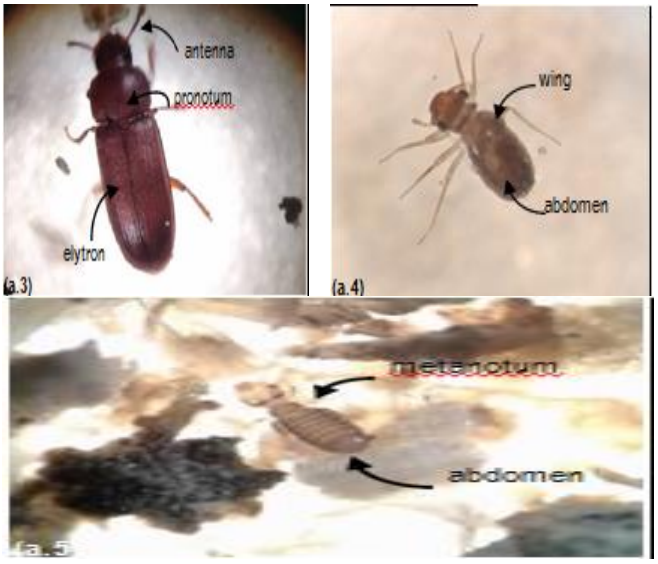
As expected, insects, mites and fungi were detected and varied in number, genus and species, as the time of breeding progressed and animals grew. Regarding *insects and mites:* they were detected live, dead, fragmented and also in their prior stages of development (pupae/larvae) (Table 1, Figures 2.a,b).

Insects - beetles (*A. diaperinus*) at adult stage and larvae were observed in the poultry litter samples throughout the breeding period (3 to 45days), increasing in number from 3 to 50 (*Control* – ND: not detected). In addition, other insects, i.e., flies - the booklices (pscocid: *Liposcelis* and *Rhyopsocus* sp.) were also detected. Inclusive, their larvae and fragments in high number (>600) /kg of poultry litter - especially from Day 21 (Table 1). The current work data is corroborated by the findings of Skov et al. (2004) which identified beetles of the species *A. diaperinus* in all samples surveyed and so by (Mian and Dhillon, 2002) that detected the same booklices genus. It is important to emphasize that beetles of *A. diaperinus* species have been reported being ingested by the animals and were detected in their pro-ventricles and gizzards *post-mortem* (da Silva et al., 2001;Segabinazi et al., 2005).That situation can lead to certain concerns, as that beetle is known a carrier of other living organisms (fungi/mites) including bacteria (Segabinazi et al., 2005).

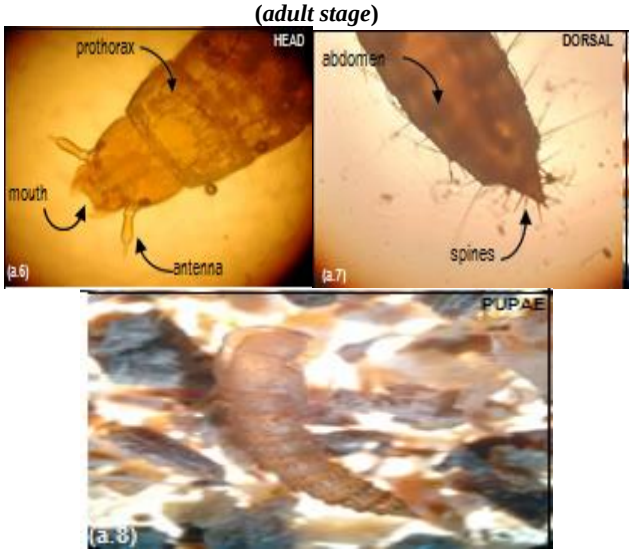
Mites - three different mites were isolated being two of them identified as *Acarus* sp. and *Dermanyssus* sp., also called white and red mite, respectively. The third was not possible to identify (Figure 2.b3). All of them were detected in quite a high number (>600/kg), since Day 3 after the chicks were transferred to the shed dry pine shavings floor (*Control*: ND) (Figures 2.b1 and 2.b2). Despite of chicken skin problem and esophagus damage (by contact and ingestion), mites act as intermediate carriers to other organisms (fungi/bacteria). The *Dermanyssus* sp. is considered one of the main poultry production pest worldwide (Kaoud, 2010; Sparagano et al., 2014). That hematophagous mite only parasites the birds during blood feeding. Most of the time it lives hidden in the shed wooden structures. Even populations of relatively small mites, that may not affect animal health through feeding (blood), can have a significant impact due to disease-bacteria related (Chauve, 1998). An example is the *D. gallinae* that has been reported being diseases vector for several infectious bacteria such as *Salmonella* (S. *gallinarum* and *S.interitides*), *Chlamydia* spp and *Escherichia coli* as well as virus such as the Newcastle (NDV– a variant of the avian *Paramyxovirus*). Important to emphasize that mites also can reach sheds and birds through mice proliferation (Valiente Moro et al., 2005). These rodents can carry and transmit mites on their bodies and legs to the birds (Valiente Moro et al. 2005; Mul and Koenraadt, 2009). Regarding their possible effects to animal well-being and health, some pathological alterations caused by mites (during blood feeding) were reported in chicken breast and legs, including feathers decay (Tucci and Guimarães, 1998). Apart from affecting animals, some allergic reactions have been reported in the chicken farming workers. Indeed, there is a high possibility of allergic diseases development in those farms working people, who develop hyper sensitivities to mites protein (Čelakovská et al., 2015).

Fungi – the poultry litter samples total fungi load increased throughout the breeding period. It ranged from $2.3x10^3$(at the 3rd day) to $1.3x10^7$UFC/g(at the 45th)as the breeding time progressed (*Control*: $2.0x10^3$). That was expected, as their growth environment conditions (temperature / moist / rich substrate) were present and increased during the whole period. In addition, there are the foreign matters incorporated (cumulated) on to the poultry litter, during chicken growth (Table 1). Through SM and SEM microscopies it was possible to visualize the fungi predominance (their conidia) on the insects dead bodies/fragmented skeletons (Figure 3.a) and the morphological structures spread throughout the pine poultry litter (Figure 3.b). The main fungus identified in the poultry litter samples was *Trichoderma* sp. which was present in all samples collected since the beginning of chicks housing. It was also registered some *field* and *storage* fungi such as *Fusarium* and *Aspergillus* & *Penicillium* which growth conditions occur at high and low humidity, respectively. Its spores were present in all surfaces, showing that, as long as they find adequate conditions to grow, they can spread fast. Figure 3.b shows *Trichoderma* sp. by SEM

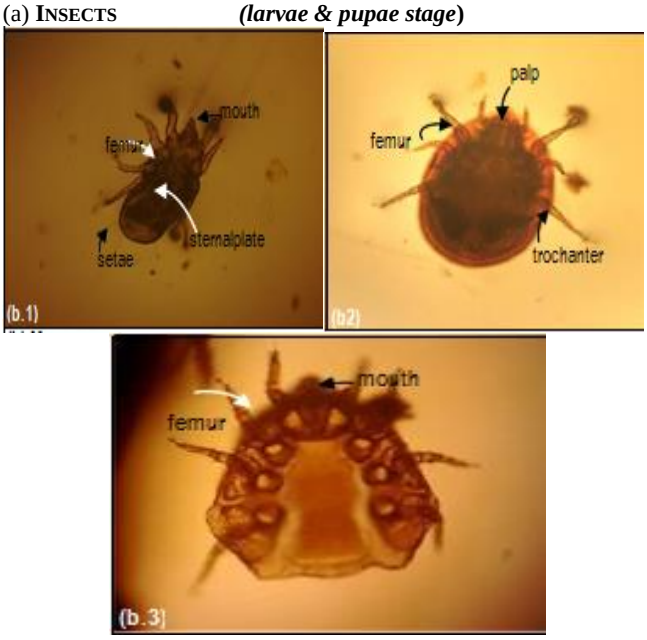




(***adult stage***)

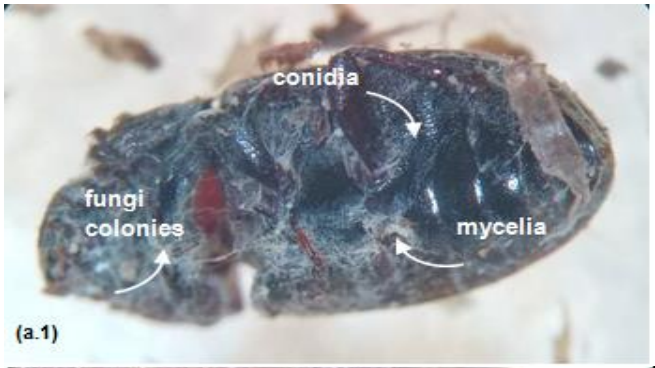


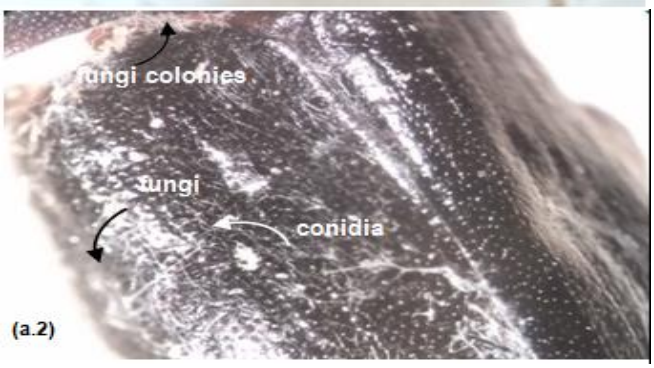
(a) **INSECTS** ***(larvae & pupae stage)***



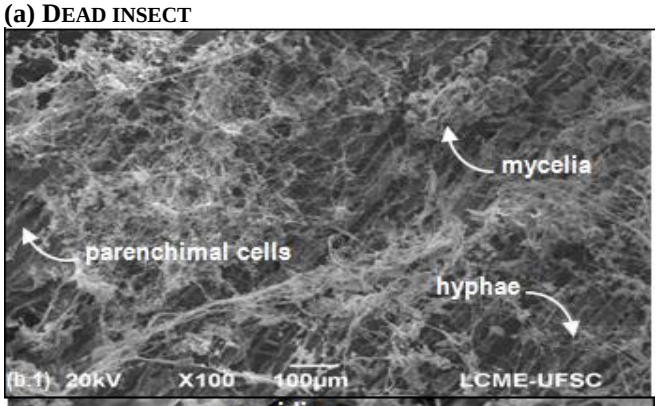
(b) MITES

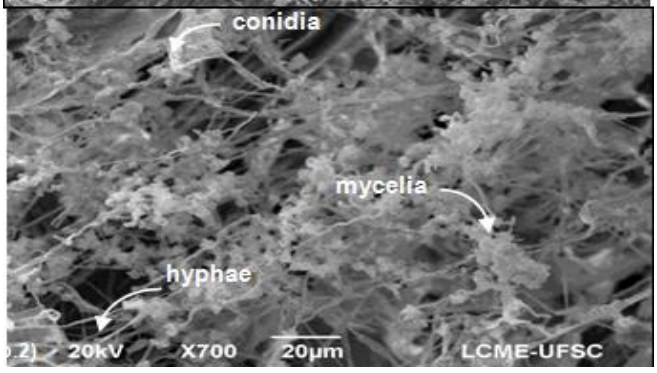
figure 2. Micrographs of **INSECTS & MITES** isolated from pine (*Pinus taeda* L.)'s poultry litter: (a.1-8) INSECTS - (a.1/a.2) *Alphitobius* sp.; (a.3) *Tribolium* sp.; (a.4) *Rhyopsocus* sp.; (a.5) *Liposcelis* sp.; (a.6/a.7) larvae *(A. diaperinus)*; (a.8) pupae and (b.1-3) MITES - (b.1) *Acarus* sp.; (b.2) *Dermanyssus* sp. and (b.3) not identified mite - by light microscopy [100x].





(a) DEAD INSECT





(b) PINE SHAVINGS POULTRY LITTER

Figure 3. Micrographs of **FUNGI** proliferation on: (a) DEAD INSECT (beetle: *A. diaperinus sp.*) on its (a.1) reverse/ under and (a.2) dorsal/back surfaces; (b) PINE (*Pinus taeda* L.) poultry litter reproductive structures of *Trichoderma* sp., by stereo [30/60 x] and scanning electron [100-700x] microscopies, respectively

B. Biodegradation changes

The physicochemical alterations of the pine poultry litter and the microscopic investigation on its cellulosic matter (tissue/fibers) decomposition characteristics, showed that the changes produced lead to optimal conditions for living organisms proliferation (Table 1, Figure 4). In addition, the foreigner matters residues that were produced and deposited onto the pine shavings (throughout the breeding stages), also

provided conditions, microorganisms proliferation, thus wood biodegradation (Figure 5).

(a) WOOD FRAGMENTS

pH - values ranged from 6.3 to 8.6, from Day zero up to the 45th, thus increasing with to the poultry litter time of use and animals growth. Similarly occurred to the titratable acidity (0.28 to 0.56%) being the highest percentage at day 21st (Table 1). Literature reports that substrates with low pH allow fungi growth, especially when happens together with optimal conditions of temperatures and humidity (mc & aw) such as pH around 5-7, mc 9-16%, a_w 0.65-0.85 and warm temperature (25- 35°C) for storage fungi. The field fungi need higher humidity (mc: up to 24-25%) (Magan and Olsen, 2004; Scussel et al., 2017). Both fungi group were detected in the current work (Section 3.1). Bacteria prefer higher pH such as achieved during the chicken breeding too (Carr et al., 1995).

Humidity - as far as aviary bed samples mc and a_w are concerned, the *(a)mc* increased**,** despite of certain reduction from the *Control* (13.9% [12.8-15.1]) that occurred at the beginning of the chicks housing up to Day 3 (it reduced to 11.1% [9.7- 12.4]). That was due to the heating applied in the shed to keep chicks warm. Mc highly increased to 34.0% (32.0-36.0) and then to 40.6%(28.9-62.3) from the 21st to 45th day of breeding. They were therefore, quite adequate for fungi, inclusive for bacteria growth. Similarly, the *(b)aw*, which at *Control* was 0.873(0.862-0.885), it lightly reduced at Day 3 to 0.740(0.738-0.744) and then increased as the breeding stages advanced and kept high, around 0.960(0.948-0.954) from the 21st day onwards.

Dimensions, *color* and *tissues* -as far as the wood fragments alterations are concerned, it was possible to observe that, as the birds grew heavier, the floor poultry litter layer got compacted and their particle sizes modified & reduced. As tissues broke down, their size reduced from: as big as 23 mm (Day zero) to smaller particulates (<1 mm).Through their microscopic characteristics, *fibers* changes were registered. They degraded along the different stages getting morphological characteristics quite away from those of *Control* (considered regular shape) (Figure 4). *Color and stains* - at Day 3 the shavings got slightly stained, and then changed from light-brown to dark-brown tones. By the time of reaching the end of breeding stages (Day 45), poultry litter samples got particles almost black in colors (with some white fungi patches).

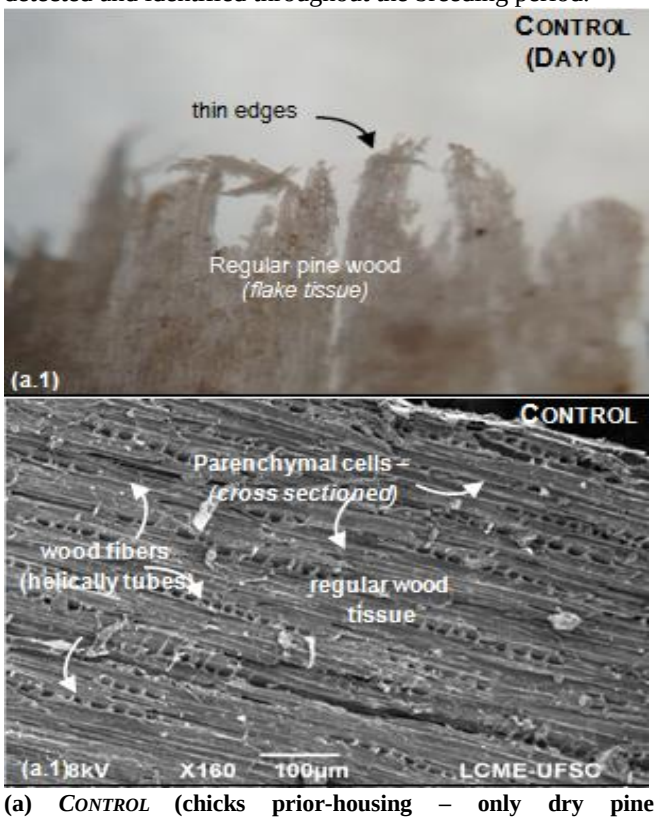
Fungi degradation - at Day 3 the wood tissue (shavings) still had its slightly regular edges (similar to *Control* - Figure 4.a). However, some particles adhered on its surface (foreigner matters/feed) was observed (Figure 4.b). No fungi wood degradation was observed, only their presence (microscopically detected). On the other hand, on day 21 the shaving edges got thinner and rather broken, showing changed color (darker brown/black) particulates and highly fungi spoiled with small particles adhered (animal faeces, feed residues and insects fragments). Following that, the shaving edges were quite damaged (broken), with very dark color particles or totally disintegrated into micro-fragments and quite a lot of highly adhered particles onto them. Indeed, their disintegration by *fungi* enzymes catalysis at this stage (celluloses and ligninases, among others) were clearly observed and registered also the presence of stains (Kreibich et al 2017). Fungi metabolites (toxins) contamination is possible at this stage, depending on whether toxigenic fungi species are present. They can get into the chicken products (manly liver/eggs) and can cause disease, even animals death or human liver cancer (Magan and Olsen, 2004; Zain, 2011).

(b) FOREIGN MATTER

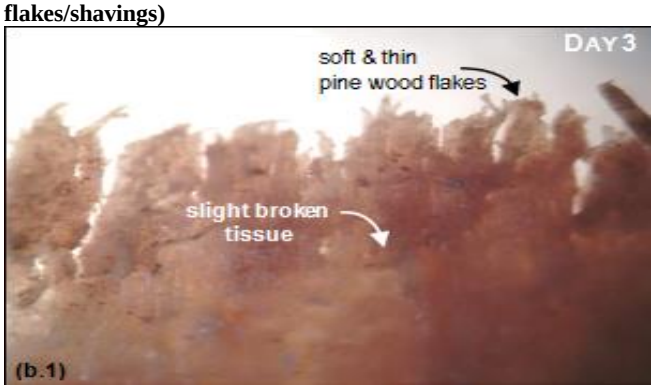
Apart from insects and other living organisms detected in the pine poultry litter, different foreigner matters (impurities) were detected. They were visualized and identified by LM and SM as animal wastes (faeces), feathers (loose and their fragments), including chicks semi-plumes (seen only microscopically) and feed residues (ground dry corn - *Zea mays* L.).

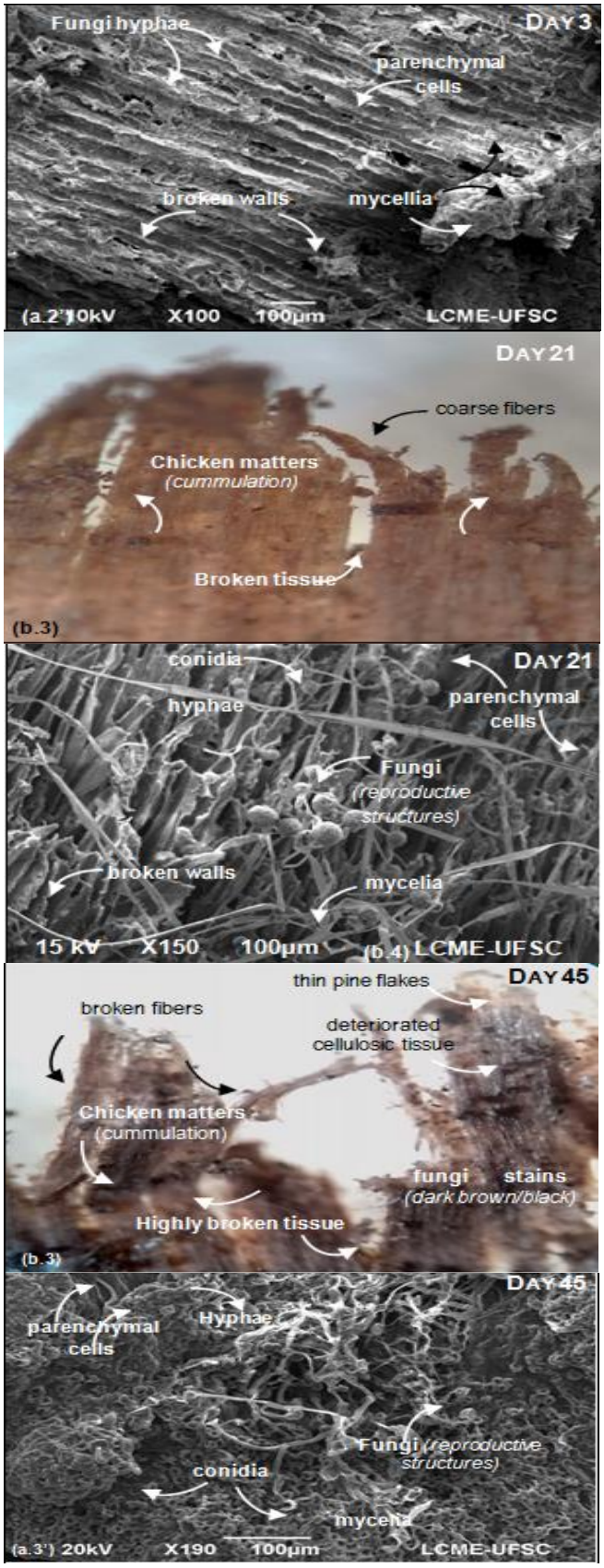
Animal wastes, *semi-plumes and feathers* - they were deposited onto shavings by the animals during the 45 days breeding. Their presence leads to acidity alteration and humidity increase, therefore adequate for living organisms proliferation (Magan and Olsen, 2004).

Feed residues - they can affect animal well-being and health due to its substrate (corn) being suitable/rich for fungi growth (as long as they find adequate humidity and temperature) thus skin mycosis and/or toxins production (affecting animal liver). Figure 5 shows the pine poultry litter foreigner matters detected and identified throughout the breeding period.



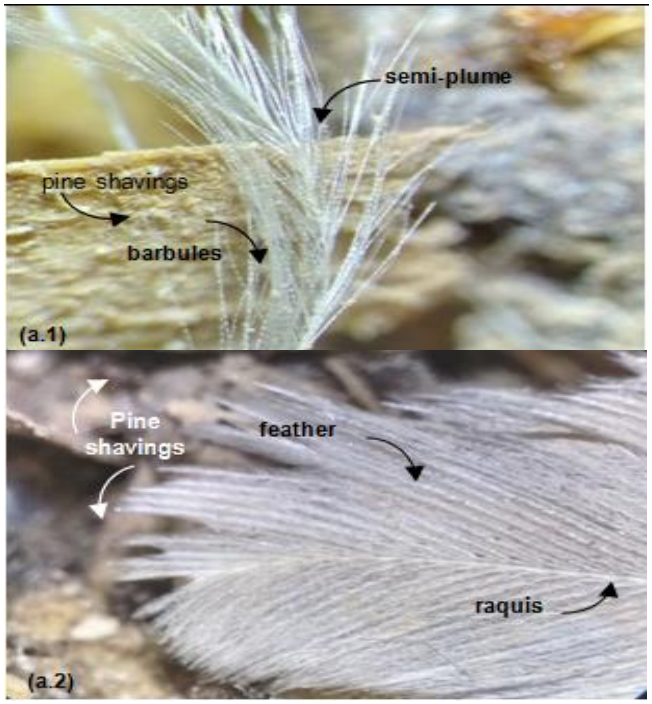
(a) *CONTROL* (chicks prior-housing – only dry pine flakes/shavings)



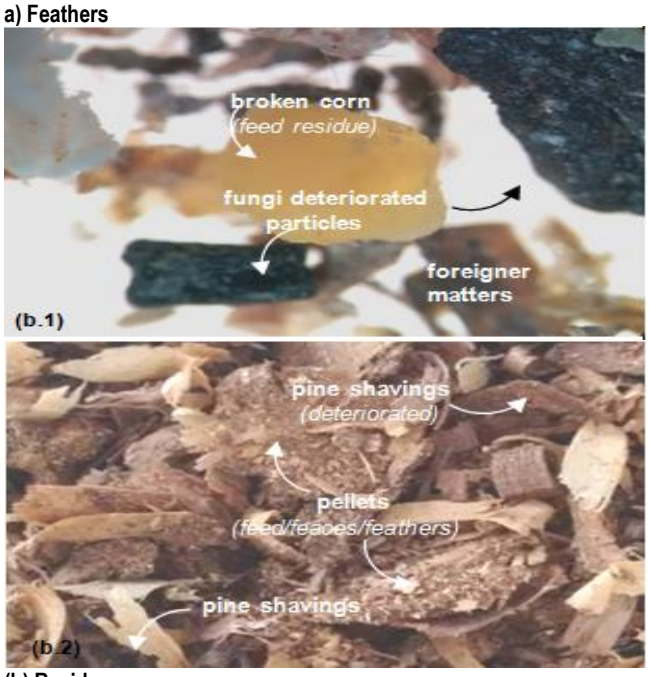


(b) POULTRY LITTER (at chicks post-housing) Day 3 to 45

Figure 4. Micrographs of (a) **DRY PINE** (*Pinus taeda* L.) **SHAVINGS** characteristics - *Control* (Day zero) and the (b) **CHICKEN POULTRY LITTER** biodegradation changes (wood edges & surface) that occurred (b.1/b.2/b.3) at 3rd, 21st and 45th days of chicken breeding period - by stereoscopy (column 1) [50x] and scanning electron microscopy [100-190x] (column 2).



a) Feathers



(b) Residues

Figure 5. Micrographs of **FOREIGN MATTERS** isolated from chicken pine (*Pinus taeda* L.) poultry litter: (a) FEATHERS - (a.1) semi-plume – from chicks, only seen microscopically (at Day 3); (a.2) feather – from adult animal (at Day 21) and (b) FEED - (b.1) residue - ground corn (*Zea mays* L.) (at Days 21 & 45) ; (b.2) pellets – mix of foreigner matters that accumulated during the 45 days of animals breeding period - by stereo microscopy [20-40x].

C. Poultry litter living organism, its cellulose material biodegradation versus

chicken well-being, health and products safety

As insects and mites are carriers of microorganisms (fungi, bacteria, virus) their presence and proliferation can lead to development of diseases such as allergies/itches, lesions/scares, mycosis, toxins syndrome (toxic effects) and viruses either, for the animals during breeding (in the sheds) and so the humans (that work in the poultry farm and live

nearby), apart from chicken liver´s consumers. Regarding bacteria, several diseases have been reported being caused by their presence in chicken during breeding (Bender, et al., 1991). Regarding fungi contamination apart from the saprophytes, also the toxigenic *Aspergillus* sp. and *Penicillium* sp., *Fusarium* sp. have been reported.

They can cause problems in the skin(mycosis) and chicken metabolism growth (due to mycotoxicosis: from moldy feed residues). They cause immune-suppression, speed growth reduction and can be transferred to the animal liver/eggs (aflatoxin B_1) and to humans through retail chicken products (Vardon et al., 2003). Toxins such aflatoxins, fumonisins, ochratoxin A among others, can be produced by those genus isolated. It is important to register that also the working people that live near the poultry farms/sheds can be affect by those insects and mites proliferation at their houses and so discomfort and develop diseases (Rimac et al., 2010).

Finally, regarding chicken well-being, they can ingest beetles and mites (fungi & bacteria infected) by picking them from the poultry litter thus exposed to possible diseases. Regarding pH, acidity & broken (small/particles) poultry litters can lead to animals scares (by contact) due to alterations of the soil physicochemical conditions - skin/scares/lesions

IV. Conclusions

The aviary environment can be a suitable place for living organisms, especially for insects and mites proliferation which can lead to health problems (cutaneous and respiratory) for the animals and so far the farming poultry workers.

Those living organisms, including deteriorating fungi in the poultry environment under high humidity (mc & a_w) and temperature (shed's and floor microclimate) conditions can be of concern due to the possibility of mycotoxin production (affecting animal and residues in meat products).

In addition, the foreign matters especially feed residues - corn) accumulation in the poultry litter together with high humidity, becomes optimal substrates for toxigenic fungi growth.

At the end of poultry breeding (45 days) the poultry litter remains as an immense residue. Usually it can be re-used as fertilizer though, as long as the living organisms are inactivated (by temperature) and no pesticides were previously (during chicken 45 days breading) applied. One thousand chicken can produce around 4 tons of poultry litter.

Acknowledgements the authors thank the CAPES (Brazilian Government grant sponsors) to C.E.S.S. and the LCME/UFSC (Central Laboratory of Electron Microscopy of the Federal University of Santa Catarina) for technical support

Table 1. Living organisms contamination and biodegradation changes of pine (*Pinus taeda* L.) poultry litter utilized during 45 days chicken breeding and their effects on animal well-being, health and meat products safety

Characteristics	Pine shavings[a] (Control)	Poultry litter contamination & changes[b]						Effects on chicken well-being & health
		Day 3		Day 21		Day 45		
		Type	Average (range)	Type	Average (range)	Type	Average (range)	
LIVING ORGANISMS CONTAMINATION								
Insects[c§]	ND[d]	Beetles[e] (L/D-W[f])	3*(NA[g])	Beetle (L/D-W/F[h])	26(W)/2	Beetle (L/D-W/F)	50(W)	Skin irritation/diseases vector
	ND	ND	NA	Booklice (L/D-W/F)	Uncountable	Booklice (L/D-W/F)	Uncontrable[Ψ]	Food unfit
Mites[§]	ND	ND	ND	Mites(w/r[ϕ])	Uncountable	Mites(w/r)	Uncountable[Ψ]	Blood/allergy/immune suppres
Fungi (TFL)[i]	2.0x10[3j]	Fungi	2.3x10[3]	Fungi	0.6x10[6o]	Fungi	1.3 x10[7]	Mycosis/toxins
Others	ND	Larvae[☆]	1	Larvae[☆☆]	6/1(w)	Larvae & pupe[☆☆☆]	4/0/40	Bacterial infection
BIODEGRADATION CHANGES								
Wood fragments pH[k*]	6.30	NA	7.35	NA	8.67	NA	8.51	Mycosis-toxins
Titratable acidity[e] (%)	0.56	NA	0.28	NA	0.45	NA	0.37	Feet scares/feathers loose
Aw[l]	0.872(0.862-0.885)	NA	0.740(0.738– 0.744)	NA	0.979(0.974–0.982)	NA	0.953(0.948-0.954)	Mycosis-toxins
Mc (%)[e*]	13.9(12.8-15.12)	NA	11.0(9.7-12.4) NA	NA	34.0(32.0-36.0)	NA	40.6(28.9-62.3)	Mycosis-toxins
Texture & condition	Rough & dry	Smooth&moist	NA	Smooth&>moist	NA	Smooth/thin&moist	NA	Thigh s/breast itching/scares
Color[r]	Wood characteristic	Light brown	0.79(1-22)	Brown	NA	Dark brown/black	NA	Discomfort
Dimension changes (mm)[m]	1.38(2-23)	NA	NA	NA	0.71(1-18)	NA	0.51(1-15)	Soil contact
Weight variation (%)[n]	34-66[o]	NA	43-57(NA) NA	NA	65-35(NA)	ND	88-22(NA)	Pathological lesion
Fiber degradation	Regular & dry	Regular & moist	NA	Broken	NA	Highly fragmented	NA	Soil contact
Fungi deterioration[p]	ND	ND	NA	FrD[q]	NA	FrD	NA	Mycosis - toxins
Stains	ND	Brownish		White&light brown	NA	White/dark brown/black	NA	Fungi growth
Foreigner matters Feed residues (%)	ND	ND[●]	<0.5	D	<0.3	D	<0.3	Mycosis-toxins
Feathers (%)[s]	ND	ND(+[s])	<05	D	<1.0	D	<2.0	Itching
Animal waste (%)	ND	D	<0.5	D	<10.0	D	<15.0	Bacteria
Pellets-impurities mix (%)	ND	ND	ND	D	<2.0	D	<5.0	Mycotoxicosis, alergies

[a] prior chicks housing - fine dry pine (*Pine taeda* L.) flakes/fragments [b] whole 45 days of chicken breeding period (number detected) [c] number of insects per kg of poultry litter (number-unity / kg) [d] not detected [e] *Tribolium, Alphitobius* + *Musca* live/dead-whole L.) [g] not applicable [h] fragment [i] total fungi load [j] UFC/g [l] color [m] only length [n] % of particle size [o] bellow (>10) and higher than (>10) than 10 mm [p] wood fragments visually deteriorated [q] fiber degradation/ deterioration [r] feather proportion to wood fagments [s] micro-feathers (semi-plume), only detected by light microscopy at Day 3 [t] inclusion of different foreigner matters, insects, mites and moist [§] microorganisms vector/carrier [Ψ] >600 [ϕ] white / red mite species *Acarus* sp. / *Deamanyssus* sp., respectively [☆] *A.diaperinus* [☆☆] *A.diaperinus* + *Tribolium* [☆☆☆] *Musca* + *Tribolium*+ *Alphitobius* * n=5 ● detected / presence

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