Isolation of some bacterial spp. in two different types of broiler litters

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Abstract:

One trial was conducted to study the microbial load or the microbiological composition of litter (red wood shavings and Rice crust), prior to placing 1-d-old chicks, and after depopulation, the microbiological composition were as following *Bacillus, Staph., Strept., Salmonella, G$^+$ rods, Enterococcus, Clostridium, E. coli, Lactobacillus ,Coliform,* the total aerobic plate count (APC) was determined and the predominant microflora of the samples was identified as possible.

Before chick placement, the APC of wood shavings (about 1.02 $\log_{10}$/g) was lower than the APC of Rice crust (about $\log_{10}$ 1.47/g) of G+),with stocking, in both types of litter the bact. contamination increased to about (5.68 $\log_{10}$ of G$^+$) and(6.76 $\log_{10}$ /g of G$^+$),respectively, no isolation of any G- bacteria before chicks placing from red wood shaving, while the count of G- bacteria was equal to $\log_{10}$ 0.69/g in the ice crust litter, and(about $\log_{10}$ 4.02 of G- and 4.95 $\log_{10}$ /g of G-), before and after depopulation, respectively with significant differences between two kinds of litter also before placing chicks and after depopulation.

عزل بعض الانواع الجرثومية من نوعين مختلفين لفرشة دجاج اللحم

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الخلاصة:

تم اجراء هذه الدراسة على نوعين مختلفين من فرشة دجاج اللحم وهي قشر الرز(السبوس) ونشارة الخشب الاحمر لعزل ومعرفة الانواع الميكروبية في انواع الفرشة قبل وبعد التربية، حيث تم تحديد العد البكتيري وتحديد بعض الانواع المعزولة مثل *Bacillus, Staph., Strept., Salmonella*G$^+$ rods, Coliform ,*Enterococcus, Clostridium, E. coli, Lactobacillus* .

كما أظهرت النتائج ان الحمل الميكروبي للفرشة قبل التربية كان ما يعادل لوغاريتم 1.02 /غم من الفرشة للنوع نشارة الخشب وكان اقل من السبوس حيث بلغ لوغاريتم 1.47/غم لبكتيريا موجبة الصبغة وعلى التوالي، وكانت النتيجة مابعد التربية هي زيادة الحمل الميكروبي بما يساوي لوغاريتم 5.68 ،6.76 لبكتيريا موجبة الصبغة على التوالي ولم يتم عزل جراثيم سالبة الصبغة قبل التربية من فرشة النشارة الحمراء وعزلت من قشرة الرز بما يعادل لوغاريتم0.69 /غم بينما ازدادت أعدادها بعد التربية بما يعادل لوغاريتم 4.02، 4.95 /غم على التوالي مع وجود الفارق المعنوي بين المجموعتين ووجود الفارق بين الأعداد الجرثومية قبل و بعد التربية.

Introduction:

Litter quality and composition play a role in bird performance and a possible source of zoonotic agents, and affect the microbial colonization of the gut of the birds there for litter is an important factor in poultry hygiene (1).

Plant residues such as straw ,Rice crust ,wood shavings, and recycled paper products are common used in poultry industry (2),the depth of litter was reported as 5 to 10 cm (3) and free from any pollutants, clear of microbial contamination specially before use.

Litter humidity and subsequently its ammonia content are important factors for skin condition (4), reduction of litter humidity and ammonia content could be achieved by ventilation of the litter floor (4,5) identified three litter conditions associated with contact dermatitis, litter moisture, greasy litter and litter nitrogen.

Microbiological composition of litter several major taxonomic groups have been isolated from litter, Enterobacteriaceae, Gram positive irregular rods, lactobacilli, micrococci/ staphylococci, strepto cocci and bacilli, also moulds and yeasts ,Clostridia were also present(16).

Terzich *et al.* (2000) examined the microbiological composition of poultry litter from 12 regions of the USA., found Gram-negative and Gram-positive bacteria,*Staphylo coccus*, *E. coli* and coliforms were present. *Staphylococcus* was most frequently identified (6). Martin et al. (1998) examined microbiological composition with respect to total bacteria, *S. aureus*, Gram-negative bacteria, E. coli O157:H7, moulds and Salmonellae in composted and non-composted litter during spring, summer and winter (7). No salmonellae or E. coli O157:H7were found at all, However, there was no influence of litter condition (new and used wood shavings) on the contamination of fully processed post-chill broilers (10), old litter (pine shavings) was supposed to control salmonellae colonization in newly hatched chickens because of the existing microflora (11), while Soerjadi-Liem and Cumming (1984) concluded that chickens acquired protective microflora from their immediate environment (12).

The aim of study is to isolate and enumerate the tow different litters microflora ordinary used in local broiler houses and any differences in microbial composition or level of contamination between rice crust and red shaving litters before and after use.

Materials and methods:

Management of birds and litter were conducted, for the experiment, 200 newly hatched broiler chicks were purchased from a commercial local hatchery and randomly divided into two groups of 100 chicks.

The stocking density was 10 birds/m^2 and they were fed a commercial chicken starter ration according to NRC(1994) (13) containing no growth-promoting antibiotics requirement, feed and water was given *ad libitum*.

Microbial analysis starting with step1 (before housing) and step2 (after housing), random samples of litter were taken from each pen weekly using sterile plastic gloves

In this study , one group was reared on rice crust the other on red wood shavings after the bedding had been put in place, it was left undisturbed for 1 weeks in the empty pens, before the first sampling.

In all samples were collected, in all cases, analysis commenced with homogenization of 10g litter in 90 ml diluents (sterile NaCl solution, 0.85%, w/v) in a stomacher, subsequently 10-fold serial dilutions were made and the APC was determined, after plating aliquots of 0.1ml (spreading technique)from the respective dilutions on plate count agar and the same quantitative procedure was done on sheep blood agar for incubated at 35C^{o} anaerobes the plates were for 24-48hr.

Colonies on plates of two adjacent dilutions were counted .

For anaerobic bacteria dilutions were made and held at 80C^{o} for 10 min. and aliquots of 1 ml were transferred in to blood agar and iron sulphite agar and subsequently incubated anaerobically for 3 days ,black colored were recorded as positive result . Incubation was performed at 35C^{o} for 48 h. From colonies which appeared different, one representative of each different colony type was taken for cultivation and identified using tryptose agar to yield total aerobic bacteria plate counts; MacConkey agar (MAC) to yield counts of Gram-negative bacteria and lactose fermenting (coliform) bacteria; Baird Parker agar (BP) to yield counts of *Staphylococcus aureus;* EMB to yield presumptive *E. coli, Salmonella* was determined by litter samples to tetrathionate broth and incubated at 35 to37C^{o} for 24 hr and then inoculated onto brilliant green agar . Comparable data from red wood shavings and rice crust were statistically calculated using the (t-test) at a probability of 5%.

Table 1.Bacterial spp. isolated from Red wood shaving

step1 (before housing)	step 2 (after housing)
Bacillus 0	3.7×10^{5}
Staph. 0.1×10^{1}	3.9×10^{2}
Strept 0	2×10^{3}
Salmonella 0	2.5×10^{2}
G^{+} rods 0.57×10^{1}	1×10^{5}
Enterococcus 0	1×10^{1}
Clostridium 0	1.4×10^{4}
E. coli 0	1×10^{4}
Lactobacillus 0	1×10^{3}
Coliform 0	3×10^{2}
Total no. $G^{+}.\log_{10}= 1.o2^{a}$ Total no.$G^{-}.\log_{10}=0$	Total no. $G^{+}.\log_{10}=5.68^{ac}$ Total no.$G^{-}.\log_{10}=4.02^{b}$

Table 2.Bacterial spp. isolated from Rice crust

step1	**step 2**
Bacillus 23×10^{1}	7.9×10^{5}
Staph. 0	4×10^{3}
Strept 1×10^{1}	3×10^{4}
Salmonella 0	1×10^{2}
G^{+} rods 0.77×10^{1}	5×10^{6}
Enterococcus 0	0.4×10^{3}
Clostridium 0	0.9×10^{4}
E. coli 0.5×10^{1}	9×10^{4}
Lactobacillus 0.12×10^{2}	1×10^{3}
Coliform 0	2.7×10^{3}
Total no.G^{+}. $\log_{10}= 1.47^{a}$ Total no.$G.^{-}$ $\log_{10}= 0.69^{d}$	Total no. G^{+}.$\log_{10}= 6.76^{ac}$ Total $G.^{-}$ no.$\log_{10}=4.95^{b}$

Results and discussion:

Measures for reduction of pathogens must embrace the whole production chain from primary production to the end product, including cleaning from the very top of the chain, sanitizing eggs, hygiene in the poultry houses, good animal husbandry practices, and also including feed hygiene, competitive exclusion or vaccination(14), consequently, litter manage- ment should be a part of pathogen reduction programs such as acidification to a pH of below 4 (15).

microbial litter composition was analyzed , identification and bacterial total count was determined in rice crust and red wood shaving samples which presented in table 1 & 2 respectively

The number of total bacteria in these samples of step one ranged between 0 of G- to 0.52×10^{1} CFU/g of G+ bacteria while samples of step two ranged between 1×10^{4} of G- to 3.7×10^{5} CFU/g of G+ bacteria isolated from red wood shaving litter , while in the rice crust litter the step one were ranged between 0.5×10^{1} of G- to 0.77×10^{1} CFU/g of G+ bacteria and step two ranged between 9×10^{4} of G- to 5×10^{6} CFU/g of G+ bacteria.

It was clear that, there is an increment in microbial counts with significant differences as bird reared at step 1 and step 2both litters , it was found that rice crust was more contaminated of both G+ and G- bacteria comparing that with red wood shaving , this may be due to quality of litters and their efficiency to absorbed water and moisture.

It is obviously clear that the microbial growth in the litter is directed to the increased contamination this agreed with previous studies , which indicate a strong increase in the first 4 weeks (16) most of the isolated microorganisms are G^{+} positive with low count of G- negative bacteria

this agreed with (7 and 8) who they obtained very few Gram- negatives, but identified Gram- positives as a major components.

The present study confirms the predominant role of Gram-positives as well as the smaller proportion of G-negatives and that agreed with (9).

The microbial composition of the gut of newly hatched chicks is influenced by the feed and contains *lactobacilli, enterobacteriaceae, enterococci, obligate anaerobes, staphylococci/ micrococci* and *bacilli(*17). Consequently, these taxonomic groups may be found in the litter as well, which fits with the occurrence in this study of sulphite-reducing anaerobic spores after stocking the chicks possible origin of these bacteria is the chicken gut.

The absence of *Clostridia* prior to placing of both litters and increased post placing this refers to that the source is chicken's intestine ,in another word the indigenous gut microflora of the birds would be built up from feeding, water, soil and then subsequently impacting on the litter microflora., the increase of clostridia in used litter has already been reported (18), this explanation may be correct with increasing in the numbers of *Lactobacillus*. All isolates were present in both types of litter, indicating that a uniform microflora would build up, independent of the type of litter. However, the time of sampling may be played a major role litter microflora, such as *Streptococci*, *Salmonella* and *Clostridia* were detected only after placing the birds.

As findings in the literature as well as from the fate of Enterobacteriaceae, members of *Salmonella* in the litter would not be expected to decline during or after stocking. However, the larger numbers of Gram-positives against Gram-negatives in general may stabilize the microbiological balance of poultry litter.

Differences between our results and previous studies might be explained by the use of different media or different conditions , from our observation during this study blood-containing agar a wider range of microbial populations in the litter would be isolated, and this agreed with (19), other authors used selective media, but this well influence the number and the kind taxonomy of isolates so the use of selective media limit the range of microbial populations isolated.

In conclusion from the experiment, 150 samples were collected and identified, most of them were Gram positive bacteria . the number of Gram-positive isolates in particular Gram-positive rods and cocci increased at that high value, whereas the number of Gram-negative isolates remained low and that the litter quality may determined the microbial loads.

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