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Full Length Research Paper

Litter mycology and the impacts of litter type and preslaughter feed withdrawal on crop bacterial community in broiler chicken

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An experiment consisting of two trials was conducted to compare the frequency of occurrence for 8 fungi in six broiler litter materials and to evaluate the impact of litter type and preslaughter feed withdrawal (PFW) on crop bactrioflora of broiler chicken at processing plant. Litter types including rice husk (RH), shredded paper (SP), barley stalks (BS), cow dung (CD), wood shaving (WS) and a mixture of all five litters (MIX) were subjected to microbial decontamination prior to trials using 1 L formaldehyde (20% solution) per each 2 cubic feet and were kept in plastic bags for a period of one week. During the study (8-weeks), a total of 273 samples were taken from various litters at biweekly intervals and examined to monitor the presence of eight fungi species in various litters. At 56d, feeders have been removed form the pens at 0, 2, 4 and 6 h prior to slaughtered birds. Various litters showed significant difference concerning total fungal contamination ($P < 0.05$). The general frequency of eight fungi species studied was significantly differed for litters of concern ($P < 0.01$). The maximum and minimum frequencies were observed for *Aspergillus sp.* and *Fusarium sp.*, respectively. The crop bacterioflora was significantly affected by duration of preslaughter feed withdrawal. The crop bacterioflora of the birds was the normal flora up to 4 h of PFW but afterward *E. coli* was appeared and started to colonize the crop. It was concluded that various litters have significant differences regarding their potential to host different fungal community and count. Shredded paper has the potential for enhancing a fungal community rich with *Aspergillus*, a common hazardous fungus. To prevent the crop of broiler from colonization by feed-borne hazardous bacteria prior to reach at processing plant, the PFW must not exceed 4 h.

Key words: Broiler chicken, crop bacterioflora, litter, fungi, preslaugther feed withdrawal.

INTRODUCTION

One prerequisite for efficient broiler production is suitable litter concerning its chemical and physical characteristics as well as microbial count. Several fungi mold and yeast species contribute in litter microflora. These myco-related organisms impose three major unpleasant impacts on bird's life through uric acid decomposition and ammonia volatilization from litter (Bacharach, 1957; Schefferle, 1965; Burnett and Dondero, 1969 and Vogels and Vander Drift, 1976), Jones FT, Hagler WM (1982), pathogenic effects on live birds and production of mycotoxins and chemicals with adverse consequences for bird's health (Arafa et al., 1979 and Arafa et al., 1982). Many investigations have described the mycology of poultry litter (Lovett et al., 1971; Bacon and Burdick, 1977 and Timothy et al., 1995). There is no conformity on the number of myco species involved in poultry litter and their population count as they are quite diverged based on litter characteristics and poultry house climate (Thi So et al., 1978; Carter et al., Chen et al. (1979), Chen et al. (1979), 1979, Chen et al. (1979); Malone et al., 1990; Lein et al., 1992).

Preslaughter feed withdrawal (PFW) has become a

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routine practice in commercial broiler production in many countries (Hinton et al., 2000). To deny broilers form accessing feed and water ahead of cooping has an obvious impact on voiding of bird's feces and to reduce the risk of cross contamination of external parts during handling, transportation and processing of broilers (Rigby and Pettet, 1980, 1981; Papa, 1991; Northcutt et al., 1997 and Khosravinai, et al., 2002). At the same time, such a starvation positively affects colonization of crop by the most hazardous feed- borne pathogens (Soneyenbos et al., 1982 and Corrior et al., 1990; Rameriz et al., 1997) . Many studies showed that crop is several times more susceptible to be colonized by pathogens than cloaca, ceca, and intestine (Hargis et al., 1995; Ramirez et al., 1997 and Corrior et al., 1999), and also more prone to rupture during processing than any other potentially contaminant part (Humphery et al., 1993 and Ramirez et al., 1997). It has been revealed that the undesirable impacts of pre- slaughter feed withdrawal are associated with prolonged starvation of birds (Byrd et al., 1998). Experimental results have clearly suggested that time is a vital issue in embracing of preslaughter feed removal (Rameriz et al., 1997).

Meat-type poultry production based on dirt floor rearing compels large quantities of litter materials. Shavings from softwoods have historically been the bedding materials of choice in the most parts of the world. Due to growing demand and development of further processing methods, the availability of wood shavings has ever more been a problem for poultry industry promoting search for alternative litter sources. The fairly inexpensive and locally available by-products such as rice husk, shredded paper, barley stalks, cow dung (Khosravinia and Abassi, 2006), leaves, corn cobs (Khosravinia, 2006), dried rumen contents, corn-based plant by products (Khosravinia and Azarfar, 2006) and Khakshir hay (Khosravinia, 2006) were found to have substantial worth as alternative litters. However, the studies on microbial contamination of such substitute litters with special reference to their impact on preslaughter feed removal are scanty.

This study consisting two trials was undertaken to compare frequency of presence for eight naturally-occurred fungi species in the six previously tried types of local and cheap alternative bedding materials (Khosravinia and Abassi, 2006) and to evaluate the effect of litter kind and preslaughter feed withdrawal on crop bactrioflora in broiler chicken at processing plant.

MATERIALS AND METHODS

Broilers and housing

Four hundred fifty day-old straight run Arian chicks were provided from a commercial hatchery and randomly allocated to 30 pens (at density of 0.09 m^2/bird) in a partially controlled, dirt floor broiler house. Each pen was equipped with one bell waterer and one tube feeder. A 23:1 h light: dark lighting regimen was used through out the experiment. Corn and soybean meal based starter (22% CP, 3100 kcal ME/kg, 1 - 14 d), grower (20%CP, 3100 kcal ME/kg, 15 – 42 d) and finisher (18.2%CP, 3100 kcal ME/kg, 43 - 56 d) diets as well as water were provided *ad libitum.* Five litter materials viz. wood shavings (WS), cow dung (CD), shredded paper (SP), barley stalks (BS), rice husks (RH) and a mixture of identical proportion from all materials (Mix) were subjected to formaldehyde decontamination (1 L 38% commercial solution per 0.054 m^3 in plastic bags over a period of one week prior to trials) and introduced into 1 × 2 m pens at an average dept of 5 cm. No litter was added or removed during 56 days of the experiment.

Treatments and data collection

Trial 1: During the study, a total of 273 litter samples were taken at biweekly intervals and monitored for presence of eight fungi species. At each evaluation period, 100 g of well mixed litter from various parts of each pen were placed in a sterile container and transferred to the laboratory. Samples were dried at 85°C over night and blended properly. A random sub sample containing 1 g of well-blended litter was distributed on Potateo-Dextroze-Agar (PDA) cultures media (Dhingra and Sinclair, 1994) and incubated at 23.5°C for 24 h. This is followed by growth of each fungus on PDA media. Subculture preparations were practiced by single sporing and hyphal tip on water agar (WA) media to purify the fungi. Purified cultures were identified based on Nelson et al., (1983) and Ellis (1990). A mercury thermometer was used for temperature measurements. Litter pH testing consisted of weighing 50 g of litter from the top surface of litter and placing in a sterile cup, where it was combined with 100 ml of sterile distilled water. The litter and water solution were mixed thoroughly and allowed to stand for 1 - 2 min. A pH reading (pH tester 1, OAKTON, Model #35624-00) was taken and recorded. Moisture was measured based on weight loss of the 50 g samples heated in oven at 105°C for 24 h.

Trial 2: At 56 days, birds from each pen were randomly divided into 4 groups and each group deprived to access feed at 0, 2, 4 and 6 h prior to slaughter. By slaughtering the birds, crop contents were removed immediately under hygienic circumstances and they were taken to microbiology lab. For each sample, 1 g of crop contents was diluted in 9 ml physiological serum. Then, 200 μL from diluted sample was poured (streak method) on nutrient agar media and incubated at 37°C for 24 h. Finally, microbial population were counted based on colonies count and dilutions made.

Statistical analysis

The General Linear Models procedure of SAS/STAT software program (SAS Institutes, 1998) was used to analyze the data from each trail separately. In the trial 1, analysis of frequency and *chi* squared test (PROC FREQ) was applied for myco-related data to determine the significant difference between frequencies of occurrence for fungi species in a particular litter. Considering all myco data, two- way classification analysis was also adopted for the fixed effects of litter type and fungus species. In trail 2, bacteria contamination data was arranged in a 4×5 factorial arrangement and subjected to ANOVA for fixed effects of litter type, preslaughter fasting time and their interaction. In all ANOVA applications, means were separated by Duncan's Multiple Range and significance was accepted at $P < 0.05$.

RESULTS

Trail 1

The contingency table of fungus × litter for frequency

Table 1. Contingency table of fungus × litter for frequency analysis of 273 samples of litter.

Fungi (sp.)	Statistics	Litters*						Total**
		RH	SP	BS	CD	WS	MIX	Num. / freq.
Mucor sp.	Frequency	14*	9	3	7	5	3	41[a]
	Cell chi square	7.56	0.1	2.46	0.001	0.08	1.73	15.02
Pencillium sp.	Frequency	12	8	12	17*	6	7	62[a]
	Cell chi square	0.31	1.48	0.11	4.11	0.8	0.67	22.71
Aspergillus sp.	Frequency	10	9	4	10	11	20**	64[a]
	Cell chi square	0.03	0.06	4.67	0.06	0.49	10.47	23.44
Geothrichum sp.	Frequency	5	18*	8	9	8	5	53[a]
	Cell chi square	1.6	5.4	0.18	0.000	0.05	1.22	19.41
Monobelpharios sp.	Frequency	2	0	5	0	1	4	12[cb]
	Cell chi square	0.00	2.37	3.96	2.02	0.27	2.51	4.4
Alternaria sp.	Frequency	1	0	0	1	0	1	3[c]
	Cell chi square	0.52	0.59	0.53	0.48	0.42	0.63	1.1
Rhizopus sp.	Frequency	1	10	15*	2	7	2	37[ab]
	Cell chi square	4.26	0.98	11.1	2.88	0.66	2.4	13.55
Fusariom sp.	Frequency	0	0	1	0	0	0	1[c]
	Cell chi square	0.16	0.19	3.86	0.16	0.14	0.15	0.37
Total***	Number	45(b)	54(a)	48(b)	46(b)	38(bc)	42(b)	273
	Frequency	16.5	19.8	17.6	16.8	13.9	15.4	100

*RH = rice husks, SP = shredded paper, BS = Barley stalks, CD = cow dung, WS = wood shavings, and Mix = proportional mix of all five litters.
**Frequency of each fungus over pooled litter data.
***Overall frequency of fungi occurrence in each litter.
a-cMeans with different superscripts within the last column differ significantly ($P < 0.05$). (a-c)Means with different superscripts within the last row differ significantly ($P < 0.05$).

analysis of 273 litter samples and results for chi squared test for frequency of occurrence of each fungus in a particular litter in terms of cell chi squared are presented in the Table 1. There was a significant frequency of *Mucor sp*., *Pencillium sp*., *Geothrichum sp*., *Rhizopus sp*. in RH, CD, SP and BS litters, respectively, compared to other fungi species ($P < 0.05$). Frequency of occurrence for *Aspergillus sp*. was significantly greater in mix litter compared with other litters ($P < 0.01$). Irrespective of litter type, the frequency of fungi species in 273 litter samples was significantly diverged with max and min occurrences for *Aspergillus sp.* and *Fusariom sp*., respectively (23.44 *vr* 0.37%; Table 1) . Frequency analysis for fungus contamination of litters using pooled data over fungi species revealed a significant difference of fungus occurrence in various litters concerned ($P < 0.05$). In general, SP and WS hosted the max and min fungi population over the 56 days of trail (19.8 *vr* 13.9 per cent of all fungi observations), respectively.

The mean surface temperature and moisture per cent in a negative association were significantly differed for six types of litter at 56 days ($P < 0.05$). No significant effect of litter type was observed with respect to pH value at the same age ($P > 0.05$; Figure 1). The total fungi frequency in various litters was inconsistently associated with litter characteristics. The non significant coefficients of correlation among this variable and litter surface temperature, moisture per cent and pH, were 0.794, -0.235 and -0.176, respectively. In detail study of association between each fungus species and litter characteristics, there was only a significant negative correlation (-0.827) between frequency of Alternaria *sp.* and litter temperature.

Trial 2

The mean crop bacterial count for a certain type of litter was not significantly differed at a specific preslaughter fasting time ($P > 0.05$; data presented in the body of Table 2). The pooled data over preslaughter fasting time were not also significantly affected by litter type ($P > 0.05$; the last column in Table 2). Despite of an inconsistent trend of enhancement in microbial count of crop contents, there was not a significant difference for plate counts at various preslaughter feed removals for a particular litter. However, analysis of plate counts for PFW over various pooled litter data revealed a significant impact of PFW duration on mean crop bactrioflora ($P < 0.05$; the last raw in Table 2). Increasing PFW time duration caused a clear cut trend of enhancement in bacterial count of crop contents. The crop bacterioflora in birds subjected to 0, 2 and 4 h PFW were normal but afterwards crop colonization was initiated by *E. coli* as a participant in normal flora but an opportunist and potentially hazardous coli form

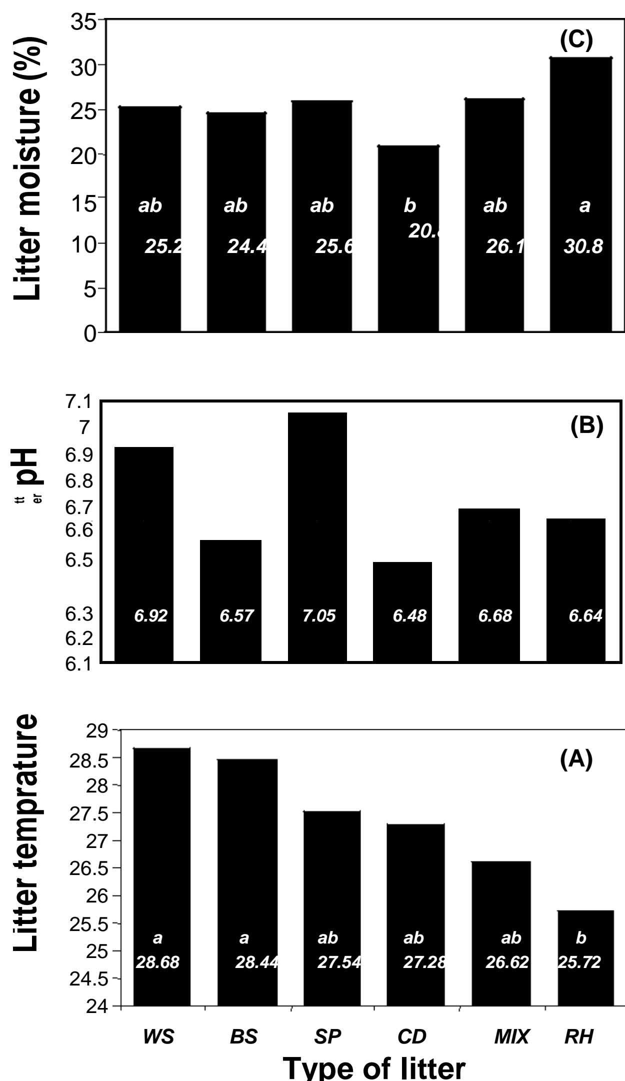

Figure 1. The trend of change in litter temperature at 2 cm depth (A), litter pH (B) and Litter moisture percent (C) at 56 day (WS = wood shavings, CD = cow dung, SP=shredded paper, RH = rice husks, BS = barley stalks and Mix = proportional mix of all five litters).

bacterium.

DISCUSSION

The eight fungi species monitored in this study are mainly among those, which have been reported by many researches. Vogels and Vander Drift (1976) listed 22 fungi species monitored in poultry litter as a few of fungi capable to degrade uric acid in poultry litter. Two species of concern in the current study, *Alternaria sp.* and *Fusariom sp.,* are not among the litter mycoflora of their study. Lovett et al. (1971) isolated 17 fungi species including *Pencillium sp.*, *Scopulariopsis sp.* and *Candida sp.* from poultry litter. They observed *Pencillium sp.* the dominant fungus in litter until it became alkaline, and then *Scopulariopsis sp.* became dominant. Bacon and Burdick (1977) found 18 species of fungi capable to grow in broiler litter, while Thi So et al. (1978) isolated 17 fungi species from various hard wood bark residues and soft wood shaving litters.

Many attempts have been made to compare different litter materials for fungi counts and diversity. Allison and Jordan (1973) and Merka et al. (1975) expressed caution regarding the use of bark residuals as litter because of possible occurrence of mycoses and production of mycotoxins due to presence of molds in this material. Veloso et al. (1974), Arafa et al. (1979), Lien et al. (1992) in a fair concord with the present study reported diverged fungal species and counts for various broiler litter materials. In the contrary, Bilgili et al. (1999) reported no significant difference in molds count of sand and pins litters (4.93 *vs* 5.08 log 10 CFU/g). However, wood fibre-based litter materials have been previously documented to contain relatively high fungal populations (Lein et al., 1992; Malone et al., 1990; Thi So et al., 1978; Carter et al., 1979). In all studies, the diverged potential of litters to host various fungi species is attributed to various litter characteristics and especially to moisture level of litter at chick placement. Significant differences of fungus occurrence in various litters concerned in the current study are in fair concord with the relevant literature as they reveal that litter source is an imperative factor in fungi diversity and population in poultry house.

The hosting capacity of a certain litter is obviously differ from others due to different physical and chemical properties at the early days of litter use. As bird's age progresses, increasing decomposition of litters that coincide with accumulation of fecal materials bring about resemblance in features of all the litters. However, the experimental results showed that even at late ages the altered litters still have distinct characteristics (Khosrvinia, 2006; Khosravinia and Azarfar, 2006), which could validate their diverged mycoflora. Minor differences in litter attributes such as temperature, caking ability, pH and moisture percent offer different circumstances to various fungi. The litter sources at late ages are still distinct in micro climates, which could be preferred either by certain or a narrow range of fungal species. In this study, six litter sources had significant differences in temperature and moisture per cent at day 56 of the experiment (Figure 1). This could cause the diverged capacities of various litters to host different ranges and populations of fungi, even though there was no significant correlation among these litter characteristics and fungus count. It also must be notified that such correlations, which have been computed based on few figures, are not reliable.

Contamination of raw poultry products by feed-borne pathogens is a serious problem all over the globe. Re-sults from several studies show improper adoption of PFW is one of the major causes for such a risk through enhancing the crop colonization by foregoing feed-borne pathogens. It

Table 2. Effect of litter type and pre-slaughter feed withdrawal time (PFW) duration (hr.) on microbial count of crop contents (log 10 CFU/ gr) at processing plant.

Litter[*]	Pre-slaughter feed withdrawal time duration (h)				Polled over time of PSFD
	0	2	4	6	
RH	8.211 ± 0.954	7.940 ± 0.413	8.761 ± 0.067	8.243 ± 0.325	8.293[a] ± 0.231
SP	8.381 ± 0.395	8.444 ± 0.105	8.534 ± 0.239	8.434 ± 0.205	8.448[a] ± 0.124
BS	8.962 ± 0.154	8.035 ± 0.675	8.645 ± 0.092	8.547 ± 0.146	8.547[a] ± 0.181
CD	6.628 ± 1.007	8.431 ± 0.140	8.267 ± 0.269	9.016 ± 0.442	8.085[a] ± 0.331
WS	6.236 ± 1.252	9.064 ± 0.581	8.872 ± 0.070	8.397 ± 0.262	7.854[a] ± 0.427
MIX	6.627 ± 1.201	8.556 ± 0.227	8.805 ± 0.059	8.491 ± 0.205	8.120[a] ± 0.347
Pooled over litter type	7.483[(b)] ± 0.395	8.244[(a)] ± 0.162	8.647[(a)] ± 0.070	8.825[(a)] ± 0.114	

[*]RH = rice husks, SP = shredded paper, BS = Barley stalks, CD = cow dung, WS = wood shavings, and Mix = proportional mix of all five litters.
[a]Means with different superscripts within the last column differ significantly ($P < 0.05$).
[(a-c)]Means with different superscripts within the last row differ significantly ($P < 0.05$).

pathogens. It has been reported that PFW facilitates the emptying of the bird's gastrointestinal tract before transportation to processing plants. It therefore decreases the amount of feces that the bird excretes during transportation (Rigby and Pettet, 1980, 1981; Papa, 1991) and in turn could reduce cross contamination of external parts of birds by ingesta. Furthermore, empting birds from fecal materials could reduce pre-mortem fecal excretion during stunning and bleeding operations in processing plant (Papa and Dickens, 1989) and reduces fecal cross contamination during evisceration operations (Wabeck, 1972). However, improper PFW may cause excessive weight loss and muscle glycogen depletion and of greater importance increases feed-borne pathogens in the crop of birds.

Increased incidence of feed-borne pathogens in crop contents is associated with tendency of the birds to consume contaminated rearing house litter during PFW (Barnhart et al., 1999; Corrior et al., 1999) . While various bedding materials have diverged potential to host various type and counts of micro organisms, it is expected that the litter source could influence the crop contamination level. The results from the current study revealed that type of litter has no significant impact on microbial counts of the crop contents (Table 2). As it has been discussed above, in correspondence with bird's age, fecal materials accumulate on the floor and reduce the proportion of bedding material in the litter. Furthermore, increasing microbial population decomposes the litters and brings all litter types toward resemblance. This common trend constricts the broad diversity on litter characteristics at the early ages of the birds to a narrow uniformity toward the end of raising period. It seems that in contrast to fungi, such a narrow diversity in litters could not be a major reason for various bacteria to inhabit in different litters. Therefore, the birds reared on various litters are exposed to bedding materials with similar microbial population during PFW. While feed deprived birds are scavenging on various litters, this could not be a source of different crop microbial count at a same PFW time duration.

However, duration of fasting period could influence the magnitude of litter foraged. The extent of litter particles picked up by bird could be a function of foraging time. The results presented in Table 2 reveal that the microbial count for the crop contents of the birds which has been slaughtered instantly after feed removal (0 PFW time) was constantly lower than longer PFW periods. Analysis of varience for fixed effect of PFW showed a significant influence of increasing PFW periods on microbial counts of crop contents.

The results of the current study with respect to appearance of *E. coli* in 6 h PFW, suggests that the PFW duration must not exceed 4 h. Byrd et al. (1998) in fair accordance with the current study observed no pathogen positive crop content samples by 2 and 4 h feed deprivation. In 5 h PFW, occurrence of Campylobacter was started and reached to maximum in 8 h. In contrast to the results obtained in this study, Rameriz et al. (1997) isolated Salmonella from the crops of broilers following 8 h of FW in a commercial rearing house. In attempt to bear out the mechanism of such crop colonization, many researchers showed that in full-fed broilers, fermentation of feed in the crop creates conditions that inhibit growth of the pathogenic micro organisms. As the crop empties during PFW, a decrease in anti-Entrobactericase activity occurs. The declined Entrobactericase activity in crop is related to decrease in lactic acid bacteria (Hinton et al., 1999), decrease in the concentration of acetic, propionic and lactic acid in the crop and increase in the pH of its contents (Barnhart et al., 1999; Corrior et al., 1999; Rameriz et al., 1997; Humphery et al., 1993).

Most of the reports emphasize that crop was several times more likely to be contaminated with Salmonella than the ceca (Corrior et al., 1999; Ramirez et al., 1997; Hargis et al., 1995). Besides crop is susceptible to rupture more frequently than the ceca or intestine during commercial processing (Humphery et al., 1993 and

Ramirez et al., 1997). Therefore, the importance of human food safety in relation with feed-borne pathogens originated form raw poultry materials urges for more emphasis on crop contamination, especially, in association with PFW.

From the results of this study it could be concluded that various litters have significant difference regarding their potential for total myco contamination. Shredded paper has a potential to host *Aspergillus* as a hazardous fungus. Preslaughter feed removal time is of more importance than litter kind with respect to its impact on crop bacterioflora at processing plant. The adoption of desirable duration of PFW time could significantly reduce the risk of pathogens occurrence in crop and consequently their transmission to raw poultry products. To avoid crop colonization by feed-borne pathogens the PFW must not exceed 4 h.

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