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Salmonella Count Changes in Fermentation of Poultry Farm Waste

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A B S T R A C T

The present study was conducted in the Division of Livestock Production and Management, Faculty of Veterinary Sciences and Animal Husbandry (SKUAST- Kashmir) to assess the *Salmonella* count changes in the poultry farm waste different seasons of fermentation. Poultry farm waste in the form of poultry carcass (dead birds) and poultry litter was selected for this purpose. Nine treatment recipes formulated for fermentation were: T_1:Poultry carcass + Poultry litter, T_2:Poultry carcass + Poultry litter + *Lactobacillus* @ 1.0 per cent T_3:Poultry carcass + Poultry litter + *Lactobacillus* @ 0.5 per cent T_4: Poultry carcass + Poultry litter + Yeast @1.0 per cent T_5: Poultry carcass + Poultry litter + Yeast @ 0.5 per cent T_6: Poultry carcass + Poultry litter + *Lactobacillus* @ 1per cent + Yeast @ 0.5per cent T_7: Poultry carcass + Poultry litter + *Lactobacillus* @ 1per cent + Yeast @ 1per cent T_8: Poultry carcass + Poultry litter + *Lactobacillus* @ 0.5per cent + Yeast @ 0.5per cent T_9: Poultry carcass + Poultry litter + *Lactobacillus* @ 0.5per cent + Yeast @ 1 per cent. At initial stage the overall highest *Salmonella* count of 8.58 log_{10}cfu was observed in T_2. At final stage, the significantly ($P<0.05$) highest *Salmonella* count of 1.08log_{10}cfu/g was observed T_1 (control group) and non-detectable *Salmonella* count was observed in all other treatments (except T_3 and T_4) during winter season. Similarly during summer season significantly ($P<0.05$) highest and lowest *Salmonella* count of 1.08 log_{10}cfu/g in was observed in treatment group T_1 (control group) and non-detectable (ND) levels of *Salmonella* count was observed in all other treatments. There was a drastic reduction in the *Salmonella* count from initial to secondary stage of composting during both the seasons. It was concluded that fermentation significantly reduces *Salmonella* bacteria in the poultry farm waste to give a secure and safe end product.

Introduction

The Indian poultry industry is the fastest growing segment of the livestock sector with 12.39 % present annual growth rate (Anon. 2015). With high levels of concentrated production, it involves generation of large volumes of waste. One of the major problems currently faced by the poultry industry is the accumulation of a large amount of waste especially manure and litter generated by intensive production (Bolan, 2010). There has been an increasing concern about the presence of infectious pathogenic bacteria in the poultry

farm waste which causes different problems both for animals as well as humans (Russell *et al.,* 1992). Poultry farm waste in the form of dead birds, offal and litter is currently being processed and utilized as feed or manure. However, the method of processing and handling of such waste results in the final product contamination (Kostrzynska and Bachard, 2006). Fermentation of poultry carcasses is a bio-secure method of disposal and utilization of poultry farm waste. It is an anaerobic process in which lactic acid bacteria transform sugar into lactic acid which is a naturally low pH effective preservative agent (Cai and Pancorbo, 1994). The lactic acid fermentation helps in decontamination of poultry farm waste to generate safe and secure final product (Crews *et al.,* 1995). The objective of this study was to determine the changes of *Salmonella* count in fermentation of poultry farm waste.

Materials and Methods

Fermentation of the poultry farm waste (dead birds and poultry litter) was carried out at Division of LPM under a roofed shed. The fermentation process was carried out in air tight plastic containers. Dead birds and poultry litter in 1:1 ratio was fermented in different combinations. Poultry waste was humidified with tap water in the proportion of 1:1 and the pH was adjusted to 6.5 with 50 % H_2SO_4 solution (El-Jalil *et al.,* 2008). A total of nine treatments (with three replicates in each treatment) with different individual as well as combination levels of culture of *Lactobacillus acidophilus* and Yeast (*Saccharomyces cerevisiae*) was used as shown in Table: 1. Dead birds and poultry litter was collected from local poultry farms. The dead birds were stored at –5^{o}C till sufficient materials were made available to fill all the containers in a single day. On the receipt of sufficient quantity of carcasses and poultry litter, the filling of fermentation containers was carried out uniformly. For *Salmonella* count changes the samples were collected at the time of loading (by mixing all the ingredients thoroughly and taking samples) and at the end of secondary stage in a serial polythene bags and sealed air tight. The samples were serially diluted in 10 fold steps using sterile triple glass distilled water. The Salmonella Shigella agar was used as selective media. The selective media was incubated aerobically for 1 day at 37ºC. The microbial numbers were expressed as $\log_{10}$ colony forming units per gram of sample (Quinn *et al.,* 1992).

Statistical analysis

The data was statistically analyzed as per the methods suggested by Snedecor and Cochran (1994). SPSS software was used for comparing the means using one way ANOVA.

Results and Discussion

At initial stage, the significantly highest ($P<0.05$) *Salmonella* count recorded was 11.33 $\log_{10}$cfu/g in T_1 (control group) and lowest *Salmonella* count recorded was 9.0$\log_{10}$cfu/g in treatment group T_6 (in which *Lactobacillus*@ 1% and Yeast@ 0.5% was added) was observed during winter season (Table. 2). Similarly during summer season significantly ($P<0.05$) highest and lowest *Salmonella* count of 11.33 and 8.25 $\log_{10}$cfu/g was observed respectively in treatment groups of T_2 (in which *Lactobacillus*@ 1%) and T_9 (*Lactobacillus*@ 0.5% and Yeast@ 1% was added) respectively. There was a significant ($P<0.05$) effect of two seasons on the *Salmonella* count in T_2 (*Lactobacillus*@ 1%), T_4 (Yeast@ 1%), T_5 (Yeast@ 0.5) and T_7 (*Lactobacillus*@ 1% and Yeast@ 1%) treatments only. The overall highest *Salmonella* count of 11.04 $\log_{10}$ cfu/g was observed in T_1 (control group). At final stage, the significantly ($P<0.05$) highest *Salmonella* count of 1.08 $\log_{10}$cfu/g in was observed T_1

(control group) and non-detectable *Salmonella* count was observed in all other treatments (except T_3 and T_4) during winter season. Similarly during summer season significantly ($P<0.05$) highest and lowest *Salmonella* count of 1.08log_{10}cfu/g in was observed in treatment group T_1 (control group) and non-detectable (ND) levels of *Salmonella* count was observed in all other treatments. The overall lowest non-detectable (ND) levels of *Salmonella* count was observed in treatment groups of T_2, T_5, T_6, T_7, T_8 and T_9. There was significant ($P<0.05$) reduction in *Salmonella* count from initial to final stage of fermentation in all treatment groups (Table. 3).

Lactic acid bacteria exhibit an antibacterial activity by using the double layer inhibition method against the different strains of *Salmonella* spp (Sakaridis *et al.,* 2012). Initially the *Salmonella* count varied significantly ($P<0.05$) between 9.0 in treatment group T_6 and 11.33 log_{10}cfu/g in treatment group T_1 during winter and 8.24 in treatment group T_9 and 11.33 log_{10}cfu/g in treatment group T_2 during summer season (Table. 2). However at the end of final stage *Salmonella* count reduced to non-detectable (ND) levels in all the treatment groups (except treatment groups of T_1, T_3 and T_4). A significantly ($P<0.05$) higher drastic reduction in *Salmonella* count was noticed from initial to final stage of fermentation (Table. 3). Although the *Salmonella* count in T_1, T_3 and T_4 was detectable but the levels recorded were significantly as low as 1.08, 0.5 and 0.41 log_{10} cfu/g respectively. Earlier Sakaridis *et al.,* (2014) reported a comparable inhibitory effect of Lactic acid bacterial on *Salmonella* count on fermented chicken carcass to non-detectable levels. Contrary to this El-Jalil *et al.,* (2008) observed only a 50 per cent reduction in *Salmonella* count due to lactic acid fermentation of poultry manure waste. Callewaert and De Vuyst (2000) found that various inhibitory substances (organic acids, diacetyl, bacteriocins, hydrogen peroxide) are generated from lactic acid bacteria which would suppress the growth of pathogenic bacteria like *Salmonella*.

Table.1 Treatment Combinations of Fermentation Experiment

Treatments	Description
Treatment 1	Dead birds + Poultry litter
Treatment 2	Dead birds + Poultry litter + lactobacillus @ 1.0 %
Treatment 3	Dead birds + Poultry litter + lactobacillus @ 0.5 %
Treatment 4	Dead birds + Poultry litter + Yeast @ 1.0 %
Treatment 5	Dead birds + Poultry litter + Yeast @ 0.5 %
Treatment 6	Dead birds + Poultry litter + Lactobacillus@ 1% + Yeast@ 0.5%
Treatment 7	Dead birds + Poultry litter + Lactobacillus @ 1% + Yeast @ 1%
Treatment 8	Dead birds + Poultry litter + Lactobacillus @ 0.5% + Yeast @ 0.5%
Treatment 9	Dead birds + Poultry litter + Lactobacillus@ 0.5%+ Yeast @1%

Table.2 *Salmonella* count (log_{10}cfu/g) due to fermentation during different stages and seasons (Mean±SE)

Treatment	Initial			Final		
	Winter	Summer	Overall	Winter	Summer	Overall
T_1	^{B}11.33±0.34	^{AB}10.75±0.24	11.04±0.15	1.08±0.04	1.08±0.04	1.08±0.01
T_2 (LB=1 %)	^{AB}9.66±0.76 ^{a}	^{B}11.33±0.37^{b}	10.49±0.13	*ND*	*ND*	ND
T_3 (LB=0.5 %)	^{AB}10.0±0.44	^{AB}9.91±0.71	9.95±0.42	1.00±0.32	*ND*	0.50±0.02
T_4 (Yeast =1 %)	^{AB}10.66±0.62 ^{a}	^{AB}9.50±0.60^{b}	10.08±0.43	0.83±0.25	*ND*	0.41±0.01
T_5 (Yeast= 0.5 %)	^{AB}9.41±0.28	^{AB}8.41±0.17	8.91±0.14	*ND*	*ND*	*ND*
T_6 (LB= 1 % + Yeast= 0.5 %)	^{A}9.0±0.46	^{AB}8.33±0.62	8.66±0.20	*ND*	*ND*	*ND*
T_7 (LB= 1% + Yeast= 1 %)	^{AB}10.33±0.19 ^{a}	^{AB}8.58±0.48^{b}	9.45±0.09	*ND*	*ND*	*ND*
T_8 (LB=0.5+Yeast= 0.5%)	^{AB}9.25±0.08	^{AB}8.83±0.66	9.04±0.04	*ND*	*ND*	*ND*
T_9 (LB=0.5+Yeast=1%)	^{A}9.16±0.25	^{A}8.25±0.38	8.70±0.12	*ND*	*ND*	*ND*

Figures with different small superscripts row wise and capital superscripts column wise differ significantly (P<0.05).

Table.3 Changes in *Salmonella* count (log_{10}cfu/g) due to fermentation from initial to final stages during different seasons (Mean±SE)

Treatment	Winter		Summer	
	Initial stage	Final stage	Initial stage	Final stage
T_1	^{B}11.33±0.34 ^{a}	1.08±0.04^{b}	^{AB}10.75±0.24^{a}	1.08±0.04 ^{b}
T_2 (LB=1 %)	^{AB}9.66±0.76	*ND*	^{B}11.33±0.37	*ND*
T_3 (LB=0.5 %)	^{AB}10.0±0.44^{a}	1.00±0.32 ^{b}	^{AB}9.91±0.71	*ND*
T_4 (Yeast =1 %)	^{AB}10.66±0.62 ^{a}	0.83±0.25 ^{b}	^{AB}9.50±0.60	*ND*
T_5 (Yeast= 0.5 %)	^{AB}9.41±0.28	*ND*	^{AB}8.41±0.17	*ND*
T_6 (LB= 1 % + Yeast= 0.5 %)	^{A}9.0±0.46	*ND*	^{AB}8.33±0.62	*ND*
T_7 (LB= 1% + Yeast= 1 %)	^{AB}10.33±0.19	*ND*	^{AB}8.58±0.48	*ND*
T_8 (LB=0.5+Yeast= 0.5%)	^{AB}9.25±0.08	*ND*	^{AB}8.83±0.66	*ND*
T_9 (LB=0.5+Yeast=1%)	^{A}9.16±0.25	*ND*	^{A}8.25±0.38	*ND*

Figures with different small superscripts row wise and capital superscripts column wise differ significantly (P<0.05).

The lactic bacteria is capable of producing and discharging into the environment a great variety of antimicrobial substances (bacteriocins), which inhibit the development of microorganisms not suitable for fermentation (Wang *et al.,* 2001).

The lactic acid and yeast fermentation of poultry farm waste yields a safe and secure fermented end product which can be utilized either as feed or manure. *Salmonella* count was drastically reduced due to acidic pH of the fermentation mixture and hence either very low or un-detectable Salmonella was found.

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