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Research Article

Determination of Optimum Pre-Slaughter Feed Withdrawal Time in Broiler Chickens and its Effect on Meat Yield, Microbiological Composition of Gut Content and Microbiological Quality of the Carcass

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Abstract: This study aimed to determine the optimum time of pre-slaughter feed withdrawal for broiler chickens to maintain profitable and hygienic meat production and to determine its effect on the meat yield, microbiological structure of gut content and microbiological quality of the carcass. A total of 100 birds were allocated to 4 identical 2 m^2 pens in a commercial broiler house, each pen housing 25 birds. The feed withdrawal durations were 18 h (group 1), 12 h (group 2), 6 h (group 3) and 0 h (group 4).

The percentage live weight loss increased as the feed withdrawal period increased ($P < 0.05$). The meat yield in group 4 was lower than that in the other groups ($P < 0.05$). Full gizzard weight in group 1 was lower than that in groups 3 and 4, and group 2 parameters were lower than group 4 parameters ($P < 0.05$).

Salmonella positive carcasses numbered 1 in group 1, 1 in group 2, 0 in group 3 and 2 in group 4.

The only significant difference found among the groups was for coliform bacteria. The coliform count in group 1 (7.54 ± 0.100) was significantly different from that in group 2 (7.00 ± 0.159) and group 3 (7.08 ± 0.169) ($P < 0.05$).

Key Words: Broiler, feed withdrawal, meat yield, carcass microbial quality

Broilerlerde Kesim Öncesi Yem Çekiminin Optimum Zamanının Et Randımanı, Bağırsak İçeriğinin Mikrobiyolojik Bileşimi ve Karkasın Mikrobiyolojik Kalitesi Üzerine Etkisinin Tespiti

Özet: Bu çalışma broilerlerde kârlı ve hijyenik et üretimi için kesim öncesi yem çekiminin optimum süresinin tespiti ve bunun et randımanı, bağırsak içeriğinin mikrobiyolojik yapısı ve karkasın mikrobiyolojik kalitesi üzerindeki etkilerinin tespiti amacıyla yapılmıştır. Toplam 100 piliç ticari bir broiler kümesi içinde her birine 25 adet olacak şekilde 2 m^2'lik 4 özdeş bölmeye yerleştirilmiştir. Yem çekme süreleri 18 saat (grup-1), 12 saat (gr-2), 6 saat (gr-3) ve 0 saat (gr-4) olarak uygulanmıştır.

Canlı ağırlık kayıp yüzdesi, yem çekme süresi arttıkça artmıştır ($P < 0,05$). Grup 4 içindeki et randımanı diğer gruplardan daha düşük bulunmuştur ($P < 0,05$). Grup 1 içindeki dolu taşlık ağırlığı grup 3 ve grup 4'ten daha düşük ve grup 2 parametreleri de grup 4'ten düşük bulunmuştur ($P < 0,05$).

Salmonella pozitif karkas sayısı grup 1 içinde 1, grup 2 içinde 1, grup 3 içinde 0 ve grup 4 içinde 2 tane olarak bulunmuştur.

Gruplar arasında bulunan tek önemli farklılık koliform bakteri içindir. Koliform bakteri sayısı açısından grup 1 (7,54 ± 0,100), grup 2 (7,00 ± 0,159) ve grup 3'ten (7,08 ± 0,169) önemli ölçüde farklı bulunmuştur ($P < 0,05$).

Anahtar Sözcükler: Broiler, yem çekimi, et randımanı, karkas mikrobiyal kalitesi

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Introduction

From a food safety point of view, contamination of a poultry carcass by gut contents has always been of prime importance as a human health threat. Contamination is also very important for poultry processors and they spend a great deal of effort to prevent contamination in their slaughterhouses (1,2). In view of the highly sophisticated machinery in use, the chances of eviscerating every bird without rupturing a single gut seem to be very small. However, not all slaughterhouses can invest in expensive machinery. Therefore emptying the gut before slaughter is gaining in importance.

On the other hand, slaughter with the gut full means a waste of feed since some of the undigested feed will be thrown away. This undigested and therefore wasted feed may also cause contamination if the gut is ruptured during processing, in turn leading to unhygienic carcasses (3,4).

It has been reported extensively that the application of a pre-slaughter feed withdrawal period will not only prevent feed wastage but also reduce the risk of carcass contamination when the gut is ruptured (5).

While a pre-slaughter feed withdrawal period helps to empty the gut, increasing the duration of the withdrawal beyond a certain period of time reduces carcass yield and giblets weight percentage over carcass weight (6).

Veerkamp (7) reported that an 8 to 12 h feed withdrawal period would be optimum but, due to some operational reasons, birds are frequently slaughtered with shorter or longer feed withdrawal times.

The objective of the study, therefore, was to re-examine the effect of pre-slaughter feed withdrawal period and its consequences on microbial quality of carcasses.

Materials and Methods

Ross-308 broilers were penned in 4 groups. Each pen measured 2 m^2. Stocking density was 12.5 birds/m^2. Groups of 25 chickens, chosen randomly from a commercial broiler flock of 10,000 birds, were placed in each pen a week before slaughter. The birds were grown under commercial conditions together with the whole flock and fed the same ration in mash form. Chickens were fed ad libitum a corn and soy based commercial ration and slaughtered at 41 days of age.

Feed withdrawal periods applied were 18, 12, 6 and 0 h for groups 1, 2, 3 and 4, respectively. Each group was separately weighed twice, firstly at the onset of feed withdrawal (LWOFW) and secondly just prior to harvest (LWPH). Group 4 was weighed only once since the moment of feed withdrawal and the moment of harvest for that group was the same. The birds were transported to the slaughterhouse in plastic live bird transport crates. The transport time was 15 min and the birds were shackled immediately upon arrival.

The birds were slaughtered following stunning in a water-bath stunner and processed according to the commercial practice. The liver, gizzard and cecum were removed after evisceration and stored in plastic bags identified by the ID number of the birds. Eviscerated carcasses were then aseptically transferred into sterile stomacher bags immediately after being cut automatically by a hock cutter and bags were identified by individual numbers for each bird. Stomacher bags containing the carcasses were then weighed.

Microbiological Analyses and Methods

Microbiological analyses were carried out in 2 steps: the determination of hygiene indicating microorganisms and *Salmonella* determination. For the determination of hygiene conditions, the following microorganisms were counted from the carcass and the cecum:

1. Aerobe Plate Count,
2. Total coliform bacteria count,
3. Enterococci count (*Enterococcus faecalis*, *Enterococcus faecium*)
4. Sulfite reducing anaerobe bacterial count.

For microbial hygiene indication, the whole carcass was transferred into a sterile polyethylene stomacher bag and diluted with 225 ml of buffered peptone water into the body cavity of the carcass. The carcass was rinsed for 1-2 min (rinse method). Then the carcass was removed aseptically and the remaining rinsate in the bags was cultured for microbiological analyses after 10-fold serial dilutions for each sample in sterile BPW, until they were diluted to 10^{-7}.

For cecum hygiene indication, the cecal content of each chicken was transferred under aseptic conditions into a sterile polyethylene stomacher bag. After recording its weight, the material was diluted 1:10 with buffered peptone water. Subsequently it was homogenized using a

stomacher for 1 or 2 min. Following homogenization, 10-fold serial dilutions for each sample were made in sterile BPW until they were diluted to 10^{-7}. The rest of the procedures for the carcass and the cecum were the same.

Each of these dilutions was inoculated into specific culture media for isolation of total aerobic plate count (8,9), coliform (8), enterococci (10), and sulfite-reducing anaerobic bacterial counts (11). The counting of these microorganisms was performed using a drop plating technique. Colonies on plates were manually counted and reported as base 10 logarithm of colony forming units per gram of sample (log 10 cfu/g).

The specific culture media for each organism and incubation conditions are shown in Table 1. All the Oxoid culture media was obtained from Unipath Ltd, Basingstoke, Hampshire, UK.

Isolation of Salmonella species from carcasses and cecum

Carcass testing. The isolation of *Salmonella* spp. was carried out in 2 enrichment steps. For this purpose, the whole carcass was transferred into a sterile polyethylene stomacher bag and 225 ml of BPW was added to the body cavity. The carcass was rinsed for 1-2 min (rinse method). Then the carcass was removed aseptically and the bag incubated at 37 °C for 24 h (8).

Cecum testing. The cecal content of each chicken was transferred under aseptic conditions into a sterile polyethylene stomacher bag. After recording its weight, the material was diluted 1:10 with buffered peptone water. Subsequently it was homogenized using a stomacher for 1-2 min, and the bag incubated at 37 °C for 24 h (8). The rest of the procedure for the carcass and the cecum was the same.

Following incubation, 1 ml of each incubated BPW was transferred into culture tubes containing 10 ml of Selenite Cysteine (12,13) and 0.1 ml of each incubated BPW was transferred into culture tubes containing 10 ml of Rappaport Vassiliadis (RV) (14) enrichment broths. SC broth was incubated at 37 °C for 24 h, and RV broth was incubated at 43 °C for 24 h. The 2 cultures were then separately streaked onto Brilliant Green Agar plates and incubated at 37 °C for up to 48 h.

The agar plates suspected of containing *Salmonella* spp. were selected, and the colonies were then identified by Gram staining and standard biochemical tests and serological tests were carried out with *Salmonella* polyvalent serum.

Statistical Analyses

Differences between the groups for all of the parameters were determined by analyses of variance (ANOVA). Parameters related to the microbiological data were transformed into logarithmic scale in order to carry out variance analyses. A least significant difference (LSD) test was carried out to determine the significance of the differences among the groups. Pearson correlation coefficients were calculated to determine the correlations among the microbiological variables (15). SPSS-11 for Windows was used (16).

Table 1. The culture media for microbiological analyses and incubation conditions.

Microorganisms	Culture media	Incubation temp. (°C)	Conditions time (h)	Anaerobe/Aerobe
Total aerobic mesophile plate count	Plate Count Agar (Oxoid CM 325)	30	48-72	Aerobe
Coliform	Violet Red Bile Agar (Oxoid CM 107)	37	24-48	Anaerobe
Enterococci (*Enterococcus faecalis, Enterococcus faecium*)	Slanetz and Bartley Medium (Oxoid CM 377)	37	24-48	Aerobe
Sulfite-reducing anaerobe bacteria	Perfringens Agar Base (Oxoid CM 543; Suppl.SR 76+77; Gas generating kit-BR 38)	37	24-48	Anaerobe

All the Oxoid culture media was obtained from Unipath Ltd, Basingstoke, Hampshire, UK.

Results

The live weight at onset of feed withdrawal (LWOFW) for groups 1, 2, 3 and 4 was 1949 ± 66.75 g, 1990 ± 54.38 g, 2023 ± 60.20 g and 1965 ± 55.30 g, respectively. The live weight prior to harvest (LWPH) for groups 1, 2, 3 and 4 was 1852 ± 63.65 g, 1918 ± 51.20 g, 1987 ± 60.51 g and 1965 ± 55.30 g, respectively. The percentage live weight differences between LWOFW and LWPH for groups 1, 2, 3 and 4 were 4.99 ± 0.18, 3.54 ± 0.21, 1.81 ± 0.16 and 0.00 ± 0.00, respectively (Table 2).

The yield values for groups 1, 2, 3 and 4 were 69.21% ± 0.00351, 68.67% ± 0.00379, 68.95% ± 0.00375 and 67.00% ± 0.00354, respectively (Table 3).

Full gizzard weights for groups 1, 2, 3 and 4 were 46 ± 1.62 g, 49 ± 1.29 g, 50 ± 1.37 g and 55 ± 1.69 g, respectively, and empty gizzard weights for groups 1, 2, 3 and 4 were 36 ± 1.11 g, 38 ± 1.12 g, 39 ± 0.89 g and 38 ± 0.99 g, respectively (Table 4).

Liver weights for groups 1, 2, 3 and 4 were 39 ± 1.49 g, 42 ± 1.26 g, 42 ± 1.25 g and 41 ± 1.41 g, respectively, and the ratio of liver weight to LWPH for groups 1, 2, 3 and 4 was 2.10 ± 0.04, 2.15 ± 0.06, 2.13 ± 0.06 and 2.14 ± 0.06, respectively (Table 4).

The general microbiological profile of carcasses is shown in Table 5 and of the ceca is shown in Table 6.

Out of the total 92 carcasses, 4 were contaminated with *salmonella* (Table 5). No *salmonella* contamination, however, was found in any of the 95 cecum samples (Table 6).

The correlations of microbiologic loads of carcasses and ceca are shown in Table 7.

Discussion

The results indicate that live weight at onset of feed withdrawal (LWOFW) and live weight prior to harvest (LWPH) did not differ significantly in each group (Table 2).

Table 2. Body weight (g).

Feed Withdrawal (FW) Groups	Live Weight at onset of FW LWOFW	Live Weight just prior to harvest LWPH	Percentage Difference
Group 1 (18 h) (n = 25)	1949 ± 66.75	1852 ± 63.65	[a]4.99 ± 0.18
Group 2 (12 h) (n = 25)	1990 ± 54.38	1918 ± 51.20	[b]3.54 ± 0.21
Group 3 (6 h) (n = 25)	2023 ± 60.20	1987 ± 60.51	[c]1.81 ± 0.16
Group 4 (0 h) (n = 25)	1965 ± 55.30	1965 ± 55.30	[d]0.00 ± 0.00

[abcd] Percentage differences associated with different superscripts differ significantly ($P < 0.05$) .

Table 3. Live weight just prior to harvest and carcass weight (g).

Feed Withdrawal (FW) Groups	Live Weight just prior to harvest LWPH	Carcass Weight	Yield
Group 1 (18 h FW) (n = 25)	1852 ± 63.65	1288 ± 49.11	[a]69.21% ± 0.00351
Group 2 (12 h FW) (n = 25)	1918 ± 51.20	1333 ± 38.58	[a]68.67% ± 0.00379
Group 3 (6 h FW) (n = 25)	1987 ± 60.51	1376 ± 48.48	[a]68.95% ± 0.00375
Group 4 (0 h FW) (n = 25)	1965 ± 55.30	1312 ± 44.12	[b]67.00% ± 0.00354

[ab] Figures in the same column associated with different superscripts differ significantly ($P < 0.05$).

Table 4. Liver and full and empty gizzard weights (g).

Groups	Full Gizzard	Empty Gizzard	Liver	
			Weight	% LWPH
Group 1 (18 h FW) (n = 24)	^{c}46 ± 1.62	^{a}36 ± 1.11	39 ± 1.49	2.10 ± 0.04
Group 2 (12 h FW) (n = 23)	^{bc}49 ± 1.29	^{a}38 ± 1.12	42 ± 1.26	2.15 ± 0.06
Group 3 (6 h FW) (n = 25)	^{b}50 ± 1.37	^{a}39 ± 0.89	42 ± 1.25	2.13 ± 0.06
Group 4 (0 h FW) (n = 20)	^{a}55 ± 1.69	^{a}38 ± 0.99	41 ± 1.41	2.14 ± 0.06

^{abc} Figures in the same column associated with different superscripts differ significantly ($P < 0.05$).

Table 5. General microbiological quality of the chicken carcasses (log_{10} cfu/g).

Carcass Groups	Total Aerobe Mesophile plate counts	Enterococci counts	Coliform count	Sulfite reducing Anaerobic Bacteria counts	Salmonella Positive carcass number
Group 1 (18 h FW) (n = 22)	8.43 ± 0.188	5.48 ± 0.161	6.36 ± 0.178	2.71 ± 0.190	1
Group 2 (12 h FW) (n = 25)	8.40 ± 0.170	5.51 ± 0.211	6.68 ± 0.106	2.73 ± 0.177	1
Group 3 (6 h FW) (n = 23)	8.13 ± 0.234	5.32 ± 0.237	6.58 ± 0.156	3.11 ± 0.236	0
Group 4 (0 h FW) (n = 22)	8.68 ± 0.215	5.51 ± 0.179	6.67 ± 0.200	2.61 ± 0.148	2

Table 6. General microbiological quality of the cecum content (log_{10} cfu/g).

Cecum Groups	Total Aerobe Mesophile plate counts	Enterococci counts	Coliform count	Sulfite reducing Anaerobic Bacteria counts	Salmonella Positive cecum content number
Group 1 (18 h FW) (n = 24)	8.48 ± 0.110	6.17 ± 0.150	^{a}7.54 ± 0.100	3.19 ± 0.140	0
Group 2 (12 h FW) (n = 25)	8.56 ± 0.106	5.96 ± 0.145	^{c}7.00 ± 0.159	3.30 ± 0.184	0
Group 3 (6 h FW) (n = 24)	8.49 ± 0.175	6.13 ± 0.204	^{bc}7.08 ± 0.169	3.29 ± 0.209	0
Group 4 (0 h FW) (n = 22)	8.59 ± 0.146	6.16 ± 0.117	^{abc}7.40 ± 0.174	2.84 ± 0.239	0

^{abc} Figures in the same column associated with different superscripts differ significantly ($P < 0.05$)

This was expected since no special care was taken with regard to either live weight or sex differences of individuals before forming the groups.

Live weight prior to harvest (LWPH) decreased in parallel to the increasing time of feed withdrawal, except for in group 3. This finding (except group 3) is supported by Buhr et al. (6), who found that live weight decreases with the increased length of time of feed withdrawal. This live weight decrease was linear ($P = 0.0002$).

The percentage live weight differences between LWOFW and LWPH among the groups were significantly different ($P < 0.05$). This indicated that the pre-slaughter feed withdrawal practice revealed its expected effect. Duke et al. (2) reported that live weight loss increased with the duration of feed withdrawal.

The percentage values of the differences between LWOFW and LWPH decreased in parallel to the decrease in the duration of feed withdrawal ($P < 0.05$). This

Table 7. Pearson correlations regarding general microbiological quality of carcass and cecum contents.

		Carcass total Aerobe Mesophile plate counts	Carcass Enterococci Counts	Carcass Coliform count	Carcass Sulfite reducing Anaerobic Bacteria counts	Cecum total Aerobe Mesophile plate counts	Cecum Enterococci Counts	Cecum Coliform count	Cecum Sulfite reducing Anaerobic Bacteria counts
Carcass total Aerobe Mesophile plate counts	r^2	1	*0.485	*0.384	*0.240	0.101	0.108	-0.191	-0.039
	P		0.000	0.000	0.021	0.345	0.312	0.073	0.716
	N	92	92	92	92	89	89	89	89
Carcass Enterococci Counts	r^2		1	*0.483	*0.406	0.126	*0.217	-0.165	0.140
	P			0.000	0.000	0.240	0.041	0.123	0.190
	N		92	90	92	89	89	89	89
Carcass Coliform count	r^2			1	*0.370	0.204	0.084	-0.112	0.040
	P				0.000	0.058	0.439	0.300	0.714
	N			90	90	87	87	87	87
Carcass Sulfite reducing Anaerobic Bacteria count	r^2				1	0.075	0.181	-0.121	0.181
	P					0.483	0.090	0.260	0.090
	N				92	89	89	89	89
Cecum total Aerobe Mesophile plate counts	r^2					1	0.175	*0.355	-0.031
	P						0.089	0.000	0.765
	N					95	95	95	95
Cecum Enterococci Counts	r^2						1	0.129	-0.039
	P							0.211	0.708
	N						95	95	95
Cecum Coliform count	r^2							1	-0.098
	P								0.346
	N							95	95
Cecum Sulfite reducing Anaerobic Bacteria counts	r^2								1
	P								
	N								95

Figures marked with an asterisk in the same column or raw are significantly correlated ($P < 0.05$).

finding is supported by Buhr et al. (6). They found that the post-feed withdrawal body weight, expressed as a percentage of pre-feed withdrawal body weight, decreased linearly with the increase in time without feed.

Yield values in group 4 only were significantly different from those in the other groups. Yield values decreased, starting from group 1, which was fasted 18 h, towards group 4, which was not fasted, with the exception of group 3. van der Wal et al. (17) reported that the oven-ready yield for full-fed broilers was about 70% to 71% of the live weights. After feed withdrawal, oven-ready yield was higher (72% to 73%) with longer deprivation. In our experiment, the yield with the longest feed deprived group (group 1) was highest (69.21%) and was smallest with the full-fed group (group 4) (67%). Significant yield improvement was obtained with a minimum of 6 h of feed deprivation. A further increase in feed deprivation improved the yield but the differences in yield for groups 1 (18 h of deprivation), 2 (12 h of deprivation) and 3 (6 h of deprivation) were not significant. The highest yield obtained with the 18 h of feed deprivation may be due to the live shrink. Bilgili (18) reported that optimum feed withdrawal time must be long enough to allow adequate gastrointestinal tract clearance, but also the shortest possible to reduce live shrink. However, we did not take live shrink into consideration in our experiment.

Full gizzard weight in group 1 was significantly different from that in groups 3 and 4; group 2 was significantly different from group 4; group 3 was significantly different from groups 1 and 4 ($P < 0.05$). Similar to our findings, a decrease in unopened gizzard weight with an increase in feed withdrawal time was reported by Buhr et al. (6). No significant differences were observed in empty gizzard weights. It has been reported that empty gizzard weights are not significantly affected by feed withdrawal time (6). According to these results, it appeared that the differences were due to the undigested feed remaining in the gizzard before passing through the gut.

Neither the differences in liver weight nor the differences in ratio of liver weight to LWPH were statistically significant between the groups. Jensen et al. (19) reported that feed withdrawal up to 8 h did not affect the liver weight significantly but increasing the period of feed withdrawal up to 16 h did significantly reduce liver weight. In our experiment, liver weight in group 1 (18 h of FW) decreased to a certain extent compared to groups 2 (12 h of FW), 3 (6 h of FW) and 4 (0 h of FW) but the decrease was not significant. In another experiment, Bartov (20) reported that 10-24 h of feed withdrawal time decreases the liver weight. Buhr et al. (6) reported that liver weight decreases in parallel as the time of feed withdrawal increases. In our experiment, the most notable decrease in liver weight was observed in group 1, in which the longest (18 h) off-feed period was applied.

The differences between the groups regarding hygiene-indicating microorganisms over carcasses (total aerobe mesophile plate counts, *enterococci* counts, *coliform* counts and sulfite reducing anaerobic bacteria counts) were not statistically significant.

While group 3 had the lowest value for total aerobe mesophile plate counts (8.13 $\log_{10}$ cfu/ml of carcass rinse), the same group had the highest value for the sulfite reducing anaerobic bacteria counts (3.11 $\log_{10}$ cfu/ml of carcass rinse). This finding might not be due to the feed withdrawal and may be explained as the result of competition among the intestinal microflora, i.e. competition between aerobes and anaerobes in the intestine and therefore sulfite reducing anaerobe bacteria possibly suppressed the aerobe microflora (21,22).

Regarding the coliform count in cecum content, the result in group 1 (7.54 ± 0.100) is significantly different than that in group 2 (7.00 ± 0.159) and group 3 (7.08 ± 0.169) ($P < 0.05$). The highest count was observed in group 1, where the feed withdrawal was longest. This condition may be expected, resulting from stress caused by the longest feed withdrawal. It was reported that there is a good balance in the bacterial population in the gut of healthy animals but this balance may be hampered by some changes that may also cause stresses, such as transportation, sudden change in ration, stocking density, extreme cold or hot conditions, humidity, vaccination and starvation. As a result of these stresses, the defense mechanism of the animals is weakened and therefore a number of the pathogen microorganisms that were suppressed earlier become active (23-25).

Lactobacilli prevent the pathogen bacteria from settling on the gut wall epithelium in competitive conditions (26). Probably due to the effect of starvation, stress may be caused in group 1, with the longest (18 h) feed withdrawal, and the protective function of the lactobacilli becomes lessened, leading to the increase in

the number of coliform bacteria in the gut. In some experimental works reported by Jin et al. (27) and Watkins and Kratzer (28), lactobacilli reduced the number of coliform bacteria in the gut. However, as we did not consider the lactobacilli in our experiment, one can postulate that the reduced number of lactobacilli in the gut microflora may lead to the coliform bacteria number increasing.

The differences in the numbers of the sulfite reducing anaerobe bacteria in the cecum are not significant among the groups; however, the counts of group 4 are remarkably the lowest (2.84 ± 0.239 $\log_{10}$ cfu/g). Since feed withdrawal is a stress factor it probably reduced the lactobacilli and other lactic acid bacteria in cecal flora, leading to the increase in the number of the sulfite reducing bacteria. This can be due to the antagonistic relationship between the lactobacilli and sulfite reducing bacteria (29). Similar to 18 h of feed withdrawal, 6 and 12 h of withdrawal can also be stress factors to a certain extent.

Despite the isolation of *Salmonella* spp. from 4 carcasses, no *salmonella* was isolated from cecal contents. This result is not because different feed withdrawal periods affect the carcasses differently. It is a reminder that carcass contamination is not only due to the rupture of the gut. It can only be explained by cross-contamination of the carcasses in the scalding tanks, in the plucker, or on the evisceration line, since there are no correlations between the carcass *Salmonella* contamination and that of cecal content. Lillard (30) showed that the *Salmonella* positive broilers at the level of 3%-4% become 35% *salmonella* contaminated carcasses after slaughter and claimed that this finding is due to cross-contamination of the carcasses in the slaughterhouse. Our experiment was carried out in a commercial slaughterhouse that had previously slaughtered some several thousand broilers from several different houses. Therefore most probably the scalding tank or plucker or evisceration line was contaminated with *salmonella* from other sources rather than our broilers and this eventually contaminated some of our broilers.

Significantly positive correlations were seen among all the parameters relating to the carcasses ($P < 0.05$). As explained earlier, the cross-contamination risk existed and occurred in the commercial slaughterhouse and the carcasses then became contaminated by the equipment. The positive correlations between the cecum coliform bacteria counts and the cecum total aerobe mesophile counts and between the cecum enterococci counts and carcass enterococci counts were significant ($P < 0.05$).

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References

1. Hinton, A.Jr., Buhr, R.J., Ingram, K.D.: Physical, chemical, and microbiological changes in the ceca of broiler chickens subjected to incremental feed withdrawal. Poult. Sci., 2000; 79: 483-488.
2. Duke, G.E., Basha, M., Noll, S.: Optimum duration of feed and water removal prior to processing in order to reduce the potential for fecal contamination in turkeys. Poult. Sci., 1997; 76: 516-522.
3. Northcutt, J.K., Savage, S.I., Vest, L.R.: Relationship between feed withdrawal and viscera condition of broilers. Poult. Sci., 1997; 76: 410-414.
4. Ramirez, G.A., Sarlin, L.L., Caldwell, D.J., Yezak, C.R. Jr., Hume, M.E. Corrier, D.E., Deloach, J.R., Hargis, B.M.: Effect of feed withdrawal on the incidence of *Salmonella* in the crops and ceca of market age broiler chickens. Poult. Sci., 1997; 76: 654-656.
5. Bilgili, S.F. Effect of feed and water withdrawal on shear strength of broiler gastrointestinal tract. Poult. Sci., 1988; 67: 845-847.
6. Buhr, R.J., Northcutt, J.K., Lyon, C.E., Rowland, G.N.: Influence of time off feed on broiler viscera weight, diameter, and shear. Poult. Sci., 1998; 77: 758-764.
7. Veerkamp, C.H.: Fasting and yield of broilers. Poult. Sci., 1986; 65: 1299-1304.
8. American Public Health Association (APHA Inc.): Compendium of methods for the microbiological examination of foods. Washington DC, 1976
9. Baumgart, J.: Mikrobiologische Untersuchung von Lebensmitten. B. Behr's Verlag. GmbH & Co.: Berlin, Hamburg, Germany, 1986.

10. Slanetz, L.W., Bartley, C.H.: Numbers of enterococci in water, sewage, and feces determined by the membrane filter technique with an improved medium, J. Bacteriol., 1957; 74: 591-595.

11. Hauschild, A.H.W., Gilbert, R.J., Harmon, S.M., O'Keefe, M.F., Vahlefeld, R.: ICMSF methods studies. VIII. Comparative study for the enumeration of Clostridium perfringens in foods. 1997; 23: 884-892.

12. Association of Official Analytical Chemists (AOAC): Bacteriological Analytical Chemists. Bacteriological Analytical Manual. 5th Ed., 1978; Washington DC.

13. June, G.A., Sherrod, P.S., Hammack, T.S., Amaguana, R.M., Andrews, W.H.: Relative effectiveness of selenite cysteine broth, tetrathionate broth, and Rappaport-Vassiliadis medium for the recovery of *Salmonella* from raw flesh and other highly contaminated foods: precollaborative study. J. AOAC Int., 1995; 78: 357-380.

14. Vassiliadis, P., Trichopoulos, D., Pateraki, E.: Papaiconomou, N., Isolation of *Salmonella* from minced meat by the use of a new procedure of enrichment. Zentralbl. Bakteriol. [Orig B.]., 1978; 166: 81-86.

15. Winer, B.J.: Statistical Principles in Experimental Design. McGraw-Hill Book Company Second Edition, 1971; pp: 149-177.

16. SPSS Inc.: SPSS User's Guide, 1999; Chicago.

17. van der Wal, P.G., Reimert, H.G.M., Goedhart, H.A., Engel, B., Uijttenboogaart, T.G.: The effect of feed withdrawal on broiler blood glucose and nonesterified fatty acid levels, postmortem liver pH values, and carcass yield. Poult. Sci., 1999; 78: 569-573.

18. Bilgili, S.F.: Slaughter quality as influenced by feed withdrawal. World's Poult. Sci. J., 2002; 58: 123-130.

19. Jensen, L.S., Cervantes, H.M., Takahashi, K.: Liver lipid content in broilers as affected by time without feed or feed and water. Poult. Sci., 1984; 63: 2404-2407.

20. Bartov, I.: Interrelationship between the effects of dietary factors and feed withdrawal on the content and composition of liver fat in broiler chicks. Poult. Sci., 1996; 75: 632-641.

21. Jay, J.M.: Intrinsic and extrinsic parameters of foods that affect microbial growth (Chapter 3). In: Modern Food Microbiology, 2003; Kluwer Academic, New York. pp: 35-56.

22. Mead, G.C., Impey, C.S.: The present status of the Nurmi Concept for reducing carriage of food-poising Salmonellae and other pathogens in live poultry. In: Elimination of pathogenic organisms from meat and poultry, 1987; Ed: Smulders, F.J.M. Elsevier, Amsterdam. pp: 57-77.

23. Fuller, R.: The importance of Lactobacilli in maintaining normal microbial balance in the crop. Br. Poult. Sci., 1977; 18: 85-94.

24. Jin, L.Z., Ho, Y.W., Abdullah, N., Jalaludin, S.: Probiotics in poultry: Modes of action. World's Poult. Sci. J.; 1997; 53: 351-368.

25. Spitz, J.C., Ghandi, S., Taveras, M., Aoys, E., Alverdy, J.C.: Characteristics of the intestinal epithelial barrier during dietary manipulation and glucocorticoid stress. Crit. Care Med., 1996; 24: 635-641.

26. Schiffrin, E.J., Brassart, D., Servin, A.L., Rochat, F., Donnet-Hughes, A.: Immune modulation of blood leukocytes in humans by lactic acid bacteria: criteria for strain selection. Am. J. Clin. Nutr., 1997; 66: 515-520.

27. Jin, L.Z., Ho, Y.W., Abdullah, N., Jalaludin, S.: Growth performance, intestinal microbial populations, and serum cholesterol of broilers fed diets containing Lactobacillus cultures. Poult. Sci., 1998; 77: 1259-1265.

28. Watkins, B.A., Kratzer, F.H.: Effect of oral dosing of Lactobacillus strains on gut colonization and liver biotin in broiler chicks. Poult. Sci., 1983; 62: 2088-2094.

29. Siriken, B., Bayram, I., Onol, A.G.: Effects of probiotics: alone and in a mixture of Biosacc plus Zinc Bacitracin on the caecal microflora of Japanese quail. Res. Vet. Sci., 2003; 75: 9-14.

30. Lillard, H.S.: Incidence and recovery of *salmonella* and other bacteria from commercially processed poultry carcasses at selected pre- and post-evisceration steps. J. Food Protect., 1989; 52: 88-91.