Effects of different mating strategies in broiler breeder during peak and postpeak phase on subsequent broiler performance

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ABSTRACT Two experimental trials on commercial broiler (Ross-308) were conducted to evaluate the carryover effect of artificial insemination (**AI**) in parent flock (**PF**) kept in cages (**C**), and on floor (**F**) in comparison to natural mating (**NM**) in floored PF. A total of 900 broiler chicks were obtained from 38-week-old PF (peak production), representing C, F, and NM evenly during first trial, whereas in second trial, similar number of chicks were obtained from same PF during postpeak phase (55 wk of age). Subsequent effects of AI and NM in PF were evaluated by bacteriology, posthatch mortality, growth performance, immune response, and carcass traits on experimental birds (broiler). Chicks being produced through NM exhibited significantly ($P \leq 0.05$) improved growth performance (feed conversion ratio, weight gain, European efficiency factor) along with the least ($P \leq 0.05$) posthatch mortality and prevalence of *Escherichia coli*, *Salmonella Pullorum*, and *Mycoplasma gallisepticum*. Moreover, the experimental chicks obtained from floored PF subjected to AI particularly during postpeak phase expressed the highest ($P \leq 0.05$) contamination of the said pathogens along with posthatch mortality. However, immune response against New Castle disease and infectious bronchitis vaccines and slaughtering parameters remained nonsignificant ($P > 0.05$) among the 3 treatments under both trials. It is concluded that the best growth performance along with the least depletion and microbial load of concerned pathogens were being pertained by the experimental birds representing NM.

Key words: broiler performance, mating strategies, parent stock, production systems

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INTRODUCTION

Ever increasing poultry population triggered the poultry industry toward cage (**C**) production system for parent flock (**PF**) to get better production since the last decade in Pakistan. Although, higher production is usually attained in C (Khan and Khan, 2018), yet it exacerbates some welfare aspects; thus, it has been banned in many developed countries (Campbell et al., 2019). While, deep-litter floor (**F**) housing is the most common and cheaper rearing system (Aviagen, 2016), but mild laxity in its care may affect the overall performance of flocks (De Jong et al., 2014; Petek et al., 2014). Most of PF are being placed on F, yet many farmers have to adopt artificial insemination (**AI**) to restrain the sharp decline in fertility after 45 wk of age (postpeak phase). According to the literature, housing systems exert regulatory sway on quantity and quality production in commercial poultry (El-Deek and El-Sabrout, 2019). The preference of AI is also applauded because of production of a greater number of chicks per hen as compared with natural mating (**NM**) (Habibullah et al., 2015). However, a major problem associated with AI in floored flock is the dirty and dusty environment loaded with several types of pathogens in poultry houses. Thus, during AI, there are chances of contamination of fertile eggs with bacteria such as *Salmonella*, *Escherichia coli* (***E. coli***), *Mycoplasma gallisepticum* (**MG**) particularly in case of careless handling in floored as well as in caged flock (Yaniz et al., 2010). Pathogenic bacteria present in the female and male reproductive tract may be another source which also can be transmitted through AI within PF as well as to their progeny (broiler) (Wang et al., 2013; Borges et al., 2014). Contrary to AI, NM is being considered a safer option particularly on F, but during copulation, some gastrointestinal tract microbes may be incorporated

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with seminal fluid (Blanco and Hofle, 2004). Resultantly chances of horizontal along with vertical transmission of certain diseases cannot be ruled out even in NM. On the other hand, trans-ovarianially and horizontally transmitted diseases of poultry are vital in determining the fate of a broiler flock (Zaheer, 2016) because such birds cannot cope up with forced growth phenomenon in commercial poultry.

Chick's hygienic status can be associated with method of insemination through which they are being produced (Li et al., 2018). As these heired contaminants not only hamper the posthatch performance but also deteriorate the meat quality that ultimately low down the consumer preference toward chicken meat (De Silva et al., 2017). Furthermore, some studied like De Reu et al., 2004; Sayyazadeh and Shahsavarani, 2005 stated that AI can interfere the egg geometry which may lead to poor quality chicks, whereas some other studies are evident that bacterial transmission is being intensified with progression of PF age (Lutful Kabir, 2010). Indeed, chick quality is a primary influential factor in regulating the growth performance of a broiler flock. Poor quality chick carries certain diseases, which ultimately lead to poor growth performance along with lower livability and eventually higher cost of production (Mitchell, 2015). Whereas, Salmonella especially Salmonella Pullorum (**SP**), *E. coli,* and MG are not only being frequently inherited but also considered the utmost cause of mortality in broiler industry (Cox et al., 2002; Tomar et al., 2006; Heidemann et al., 2018). *E. coli* and Salmonella cause omphalitis which is being regretted as a vital cause of mortality in the first few days of chicks (CPRC, 2018). Moreover, *E. coli* and Salmonella propagate in the intestine of newly hatched infected chicks received during embryogenesis, and this infection spreads rapidly from chick to chick in the hatchery and during brooding. Although, the importance of AI cannot be ignored as an efficient and obligatory tool in caged flock and to curtail the declining of fertility in PF in floored flock, but its retro hygienic impact in broiler is yet to be unveiled in comparison to NM when conducted in caged as well as in floored PF, because quality is the vital milestone to be achieved in commercial poultry. Thus, this study has been designed to understand the myth of carry-over intrusive impact of AI vs. NM on broiler performance when the PF is being kept in different production systems (F and C) during its peak and postpeak production phases.

MATERIALS AND METHODS

The present study was carried out in collaboration of Pakistan Poultry Association North zone and University of Veterinary and Animal Sciences, Lahore, Pakistan. During this study, 2 experimental trials on commercial broiler were conducted to monitor the carryover effect of various strategies of AI and NM in PF under 2 production systems (C and F). The birds were reared under experimental animal care procedures approved by the Ethical Review Committee (No. DR/757) of the University of Veterinary and Animal Sciences, Lahore. A total of 900 commercial broiler chicks (Ross-308) were taken from a PF of 38 wk of age (peak production) during first trial, whereas, in second trial, same number of broiler chicks were obtained from postpeak phase (55 wk age) of that PF. The PF was further subjected to AI at F and C production systems while NM on F was considered as control. From each treatment (C, F and Control group) 300 chicks were placed under completely randomized design. Each treatment was comprised of 6 replicates having 50 birds each (12 birds/m^2). Three feeding regimens according to age requirement (starter, grower, and finisher) were provided *ad-libitum* to all birds according to recommendation of nutrition guide book by Ross (Aviagen, 2018). The chicks were maintained in 18 F pens of dimension (2m × 2m) on a deep litter system with rice husk as bedding material. Each pen was furnished with 2 round manual feeders and 4 nipple drinkers for *ad libitum* feeding and availability of clean, fresh drinking water. Brooding temperature and relative humidity were maintained at 32 ± 1.1°C and 65 ± 3%, respectively for the first week after hatching, after which, temperature was reduced according to formula [32– (0.32 × Age of flock)] until it reached 24°C with minimum 60% relative humidity. Organic acids and chlorination in drinking water were used instead of any antibiotics throughout the flock except vaccine's day. While, 20 Lux light of LED was provided for a period of 20 h in a day (4 h dark period). All necessary husbandry practices were followed including prescribed vaccine schedule.

Parameters Evaluated

Bacteriology. For bacteriology, 30 birds from each treatment (5 from each replicate) were picked up randomly and were slaughtered for sampling and postmortem at 0, 15th, and 30th D of age. Growth of *E. coli* was assessed by using eosin methylene blue (Levine M, 1918) selective medium, whereas plat agglutination test was used for SP and MG (OIE, 2018). At the initial stage (day 0), samples were taken from yolk and then from liver (15th and 30th D).

Posthatch Mortality in chick room and transit mortality. After hatch, experimental chicks of all 3 treatments were shifted from hatcher to chick room for grading and packing. Mortality of chicks in chick boxes (100 chicks/box) representing all 3 treatments was recorded during the stay in hatchery for period of 16 ± 2 h. Then transit mortality of same boxes in vehicles was also recorded from hatchery to commercial farms. A total 16 hatches, 8 from each phase (peak and postpeak) were monitored for data.

Mortality %

Mortality percentage was calculated as the number of birds died relative to the total number of birds multiplied by 100. Mortality was recorded on daily basis while

livability% was calculated on weekly and at the end of flock.

Growth Performance

The data were collected regarding growth performance included feed intake, live weight gain (**LWG**), weekly average weight, feed conversion ratio (**FCR**), and finally European efficiency factor (**EEF**). Feed consumption and LWG were recorded on weekly basis. Feed intake was measured as the difference between total feed offered and feed refused, and LWG was obtained by subtracting the initial body weights from the final body weights. Feed conversion ratio was measured as the ratio between total feed consumed and LWG. Birds were observed twice daily, and the dead ones were removed, and postmortem of dead birds was carried out. Finally, growth performance was gauged with EEF, which was derived by the formula (EEF = L% × Average body weight × 100 ÷ FCR × day at market).

Immune Response

Hemagglutination inhibition test was used for estimation of immune response against New Castle Disease (**ND**) and Avian Influenza (H-9) (Rubbani et al., 2001), where ELISA (Lequin, 2005) was performed to gauge immunity against infectious bronchitis (**IB**) vaccine at day 0, 15, and 30.

Slaughtering Parameters

At the end of trial, 3 birds per replicate nearest to the average weight of the same replicate were randomly picked up, kept off-feed for 4 h, and then slaughtered according to Halal standards, allowing bleeding for approximately 3 to 4 min. Carcasses were defeathered and eviscerated; carcass yield and breast, thigh, liver, gizzard, heart, spleen, and intestine relative weights were determined as percentages of live weight.

Statistical Analysis

Effects of mating strategies of broiler breeder under 2 production system during peak and postphase were analyzed on broiler bacteriology, posthatch mortality, overall livability, growth performance, immune response, and slaughtering parameters using analysis of variance technique. A completely randomized design was employed, and general linear model was used in SAS software (version, 9.1; SAS Institute Inc., Cary, NC). Data regarding peak and postpeak were analyzed by independent t test. For bacteriology and immune response, effect of age (days 0, 15, and 30) and mating strategies (AI on F, C, and NM) were analyzed separately, whereas for posthatch mortality, overall livability, growth performance, and slaughtering parameters, only mating strategies were analyzed through one-way ANOVA technique, and significant treatment means were separated by using Fisher's Least Significant

Table 1. Effect of different mating strategies on progeny bacteriology (% of positive samples out of 30 slaughtered birds each).

		Peak phase				Postpeak phase			
	Age	AIF	AIC	NM	P-value	AIF	AIC	NM	P-value
E. coli	0	$26.60 \pm 0.43^{c,x}$	$20.0 \pm 2.88^{c,y}$	$13.30 \pm 0.57^{b,z}$	0.0081	$33.33 \pm 0.58^{c,x}$	$23.33 \pm 0.69^{c,y}$	$16.66 \pm 0.59^{c,z}$	<0.0001
	15	$36.66 \pm 0.60^{b,x}$	$30.0 \pm 2.88^{b,y}$	$16.70 \pm 0.33^{b,z}$	<0.0001	$40.00 \pm 1.15^{b,x}$	$33.33 \pm 1.15^{b,y}$	$26.66 \pm 1.15^{b,z}$	<0.0001
	30	$46.70 \pm 0.57^{a,x}$	$36.66 \pm 5.77^{a,y}$	$20.00 \pm 5.77^{a,z}$	0.0195	$50.0 \pm 1.73^{a,x}$	$40.00 \pm 0.58^{a,y}$	$30.0 \pm 0.58^{a,z}$	<0.0001
P-value		0.0022	0.0034	0.0092		0.0026	0.0016	0.0044	
SP	0	$6.66 \pm 0.45^{c,x}$	$3.33 \pm 0.34^{c,y}$	$3.33 \pm 0.20^{c,y}$	0.0011	$26.70 \pm 1.15^{b,x}$	$16.70 \pm 0.69^{c,y}$	$6.66 \pm 0.59^{c,z}$	0.0026
	15	$13.30 \pm 0.51^{b,x}$	$6.66 \pm 0.20^{b,y}$	$6.66 \pm 0.40^{b,y}$	<0.0001	$28.23 \pm 0.58^{b,x}$	$26.70 \pm 1.15^{b,x}$	$13.30 \pm 1.15^{b,y}$	0.0040
	30	$26.66 \pm 0.46^{a,x}$	$16.70 \pm 0.4^{a,y}$	$13. 30 \pm 0.40^{a,z}$	<0.0001	$48.00 \pm 1.1^{a,x}$	$40.00 \pm 0.58^{a,y}$	$26.66 \pm 0.58^{a,z}$	0.0001
P-value		0.000	0.0011	0.0011		0.0033	0.000	0.001	
MG	0	3.33 ± 0.45^{c}	3.33 ± 0.45^{c}	3.33 ± 0.45^{c}	0.196	16.70 ± 1.15^{c}	16.66 ± 0.58^{b}	13.33 ± 0.58^{c}	0.1235
	15	$16.70 \pm 0.57^{b,x}$	$6.66 \pm 0.57^{b,z}$	$13.33 \pm 0.57^{b,y}$	<0.0001	$26.70 \pm 0.58^{b,x}$	$23.33 \pm 0.52^{b,y}$	$20.00 \pm 0.58^{b,z}$	0.0002
	30	26.70 ± 0.57^{a}	20.00 ± 2.88^{a}	20.00 ± 2.88^{a}	0.4008	$40.00 \pm 1.1^{a,x}$	$33.33 \pm 0.58^{a,y}$	$26.66 \pm 0.58^{a,z}$	0.0010
P-value		0.0011	0.0011	0.0018		0.000	0.000	0.000	

[a–c]Superscripts on different means within column differ significantly among different ages at $P \leq 0.05$.
[x–z]Superscripts on different means within row differ significantly among different mating strategies at $P \leq 0.05$.
Abbreviations: AIF, artificial insemination in floor flock; AIC, artificial insemination in caged flock; MG, Mycoplasma gallisepticum; NM, natural mating in floor flock; SP, Salmonella Pullorum.

Difference test. Following mathematical model was applied:

$$Y_{ij} = \mu + \tau_i + \varepsilon_{ij}$$

Where:

Y_{ij} = Observation of dependent variable recorded on i^{th} treatment

μ = Population mean

τ_i = Effect of i^{th} day or mating strategy (i = 1, 2, 3)

ε_{ij} = Residual effect of j^{th} observation on i^{th} treatment NID $\sim$ 0, σ^2

RESULTS AND DISCUSSION

Bacteriology

The prevalence of pathogens (*E. coli*, SP, and MG) was found the highest ($P \leq 0.05$) in experimental birds representing as the progeny of AI in floored and caged PF, respectively, as compared with NM having the least pathogenic load throughout the experimental life (0, 15, and 30 D) during peak phase (Table 1). The heired contamination was increased significantly ($P \leq 0.05$) with progression of age particularly in experimental birds being produced through AI. The magnitude of pathogenic load was observed to be more severe in chicks obtained from PF which was at postpeak phase in Trial 2. According to collective results of both trials (1 and 2), significantly ($P \leq 0.05$) higher incidence of subjected pathogens was exhibited by experimental birds taken from postpeak as compared with peak phase of PF. It was revealed from postmortem findings of dead and slaughtered birds that the symptoms of *E. coli* and SP were more obvious since the day 1 and airseculitis by MG in later stages in experimental birds representing AI in floored and caged PF, respectively, whereas the least one in broiler of NM. Additionally, *E. coli*, was found more obstinate and extensive on postmortem in broiler of AI in older floored flock.

These findings indicated that the most appropriate insemination method is NM to curb the pathogenic load in offspring obtained from parents of different age groups particularly when PF is being kept on floor. However, AI in caged flock was found safer as compared with AI in floored flock, but it is hygienically not better than NM, as the least growth of concerned microbes was observed in experimental birds which were representing NM (Table 1). These results are in line with the findings of Ahmed et al. (2015) who associates some link of AI with disease transmission. The elevated posthatch mortality (Table 2) and extensive numbers of positive samples of subjected bacteria (*E. coli*, SP, MG) in broiler of AI under both production systems are indicative that AI might be a contributory factor in contaminating the fertile eggs and reproductive tract of PF leading to yield the infected chicks (Table 1), which corresponds to the work of some other scientists (Corrier et al., 1999; Dhama et al., 2014). Vertical transmission of concerned bacteria might be linked with insemination techniques as yolk of DOC produced through AI possessed more infection of *E. coli*, SP, and MG in air sacs, and findings are in line of some earlier work (Reiber et al., 1995; Cole et al., 2004). Therefore, relatively hygienically compromised chicks would be produced through AI, which might be a cause of early chick mortality, and survived experimental birds would have remained under stress throughout their life. Probably, during AI, semen and reproductive tract would have been exposed to various contaminants including the subjected pathogens that originated the diseases such as Colibacillosis, SP infection, and chronic respiratory disease in the progeny/broiler (Cox et al., 2002; Tomar et al., 2006). Infected microbial enrich microclimate of the poultry house especially litter might be a major contributory factor of high microbial load in chicks.Similarly, The poor chicks quality by AI in floored flock might be because of its bedding material (litter), which served as reservoir of many a pathogens especially of SP, *E. coli* and MG.

Moreover, the handler's negligence along with pooling of semen during semen collection might have led to topsy turvy in hygienic measures during execution of AI. Similar findings were also reported by some scientists like Senthilkumar et al., 2003 and Dhama et al., 2014 where they have attributed some role of AI in the spreading of diseases. Contrarily, some other scientists gave lesser importance to whole process of AI, rather they adsorb the curse of contamination with it only in case of improper handling particularly in C reared flocks (Guy et al., 1995; Lombardo, 1998; Lierz, 2013).

Likewise, chick of older PF manifesting higher quantity of pathogens quality might be because AI would have been continuously facilitating the proliferation

Table 2. Effect of different mating strategies on posthatch mortality in chick room and transit mortality.

	Posthatch mortality (16 ± 2 h after hatch)			Transit mortality		
Treatment	Peak phase	Postpeak phase	*P*-value	Peak phase	Postpeak phase	*P*-value
AIF	0.150 ± 0.052	0.191 ± 0.041	0.842	0.183 ± 0.028	0.204 ± 0.500	0.773
AIC	0.127 ± 0.031	0.164 ± 0.058	0.270	0.160 ± 0.048	0.190 ± 0.052	0.835
NM	0.116 ± 0.032	0.130 ± 0.032	0.206	0.135 ± 0.046	0.128 ± 0.058	0.757
P-value	0.161	0.233		0.116	0.660	

Abbreviations: AIF, artificial insemination in floor flock; AIC, artificial insemination in caged flock; NM, natural mating in floor flock.

Table 3. Effect of different mating strategies on progeny livability % (0–35 D).

Treatment	Peak	Postpeak	*P*-value
AIF	92.86 ± 2.15^{c,x}	89.44 ± 1.34^{c,y}	0.044
AIC	94.03 ± 0.28^{b}	92.20 ± 1.12^{b}	0.984
NM	95.27 ± 2.13^{a}	94.80 ± 0.87^{a}	0.058
P-value	0.004	0.004	

$^{a-c}$Superscripts on different means within column differ significantly among different treatments at $P \leq 0.05$.

$^{x-y}$Superscripts on different means within row differ significantly between peak and postpeak phase at $P \leq 0.05$.

Abbreviations: AIF, artificial insemination in floor flock; AIC, artificial insemination in caged flock; NM, natural mating in floor flock.

and intensification of contaminants among the PF (Trial 2).Increasing trend in terms of quantity and quality of all 3 bacteria among all groups of experimental birds indicated that horizontal transmission's magnitude depends on inherited hygiene status to a large extent (Table 1).

It seems obvious from the findings that contamination could not be gotten rid of completely even in process of NM (Gallardo et al., 2011), as the bacterial growth in yolk of DOC from NM is quite indicative of its limitation regarding sterile chick production. Actually, during copulation, the digestive tract microbes would be incorporated with insemination fluid, which might be the reason of contamination even in broilers of NM (Blanco and Hofle, 2004; Kabir, 2010). So, it is clear indication that the insemination techniques along with production systems have a profound effect on pathogenic transmission from PF to newly hatched chicks (Li et al., 2018).

Posthatch Mortality in Chick Room and Transit Mortality

Posthatch mortality (16 ± 2 h) was found nonsignificant among all treatments; however, numerically it was clearly indicated that chicks produced from AI in both production systems (F and C) have higher mortality rate as compared with NM. The mortality pattern was similar in chicks of peak and postpeak phase, but gravity of difference in prompt posthatch mortality was deep in chicks of older floored and caged PF flocks subjected to AI (Table 2). Similarly, more mortality was also observed in the same kind of chicks when transported to commercial poultry farms in both trials (Table 2). Furthermore, these birds exhibited poor livability at the farm as mentioned ahead in Figure 1. These results reinforce the earlier discussion which was made on the basis of Table 1. The mortality pattern in chick room, during transportation and in first week of age, might have legitimated the logic that somehow AI has exerted adverse effects on chick quality. It might be by breaching hygienic status somewhere during AI which would have not let the experimental birds to combat the life's debacles particularly in chicks of floored flock. This logic is in line with views of Lutful Kabir (2010). Artificial insemination might have exposed the embryo to multiple infections especially with *E. coli*, SP, and MG (Table 1), and such pathogens would have accelerated the posthatch and transit mortality, particularly in progeny of floored flock (Table 2) (Buhr et al., 2005). Salmonella, *E. coli,* and MG can be easily transmitted vertically during AI through transovarian route as per findings of Donoghue et al., (2004) as compared with NM. While, *E. coli* and Salmonella reside and proliferate rapidly in the gastrointestinal tract of newly hatched infected chicks, and infection can spread rapidly from chick to chick in hatchery which lead to omphalitis that is major cause of early chick mortality. As the microclimate of poultry house during the process of AI was enriched with pathogens that might have been incorporated at any stage and spread to chick, which ultimately led to diseases such as colibacillosis, salmonellosis and chronic respiratory disease in the progeny/broiler causing higher posthatch mortality in birds being produced through AI (Cox et al., 2002; Tomar et al., 2006).

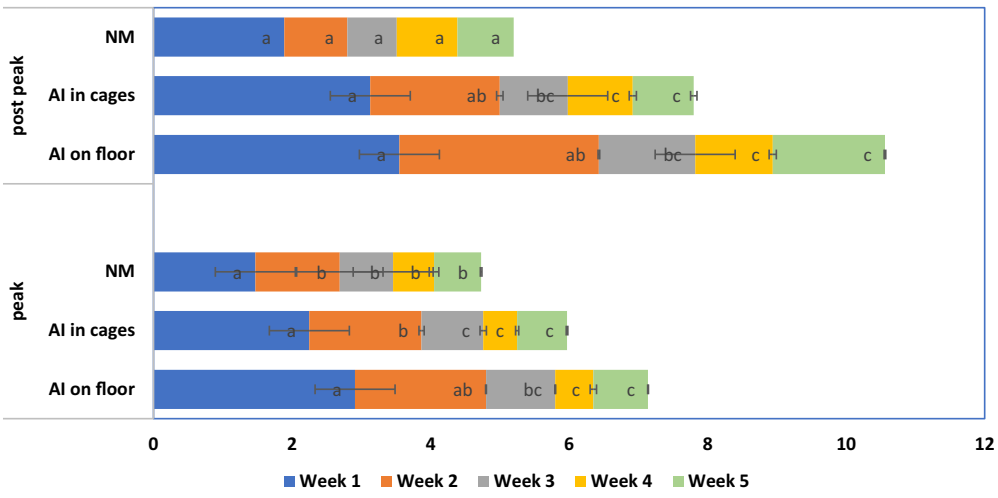

Figure 1. Trend of weekly mortality pattern in experimental birds. Abbreviations: NM natural mating; AI, artificial insemination.

Depletion %

Depletion % was recorded the highest ($P \leq 0.05$) in the experimental birds being produced by AI in floored and caged flocks, respectively, particularly during first 2 wk of age (Figure 1). While, the least depletion was found in the chicks of PF subjected to NM (Figure 1). While in second trial, broiler birds obtained from older PF (58 wk of age) for all 3 treatments suffered relatively higher mortality% than of birds taken from younger PF (38 wk) (Trial 1). Among the treatments, chicks produced through AI in floored flock during postpeak experienced the highest ($P \leq 0.05$) mortality especially in early 2 wk of age (Figure 1). However, after 3 wk of age, mortality % was recorded almost alike among all 3 competitive treatments (Figure 1). While, the best overall livability % ($P \leq 0.05$) was recorded in the experimental chick of NM (Table 3).

Conclusively, it is imperative from the data of both trials that depletion was the least in experimental broiler flock representing NM as compared with chicks produced through AI in caged and floored flocks, respectively (Figure 1 and Table 3).

It is revealed from the results of both trials that insemination method can influence the chick quality which is already been studied by (Li et al., 2018). Artifical insemination may lead to yolk sack infection (**YSI**) which has been reported the most frequent cause of poor chick quality. While, the origin of YSI may be traced back to microbial contamination of eggs of PF by mainly *E. coli* and Salmonella, which might have led to early chick mortality in experimental chicks of floored and caged flock, respectively (CPRC, 2018). Comparatively, higher early chick mortality along with greater occurrence of bacterial growth from DOC (Table 1) fixed some provoking role of AI in embryonic contamination which would have led to YSI which would have led to higher mortality in early age of experimental birds being produced through AI particularly in floored flock. Other reasons of depletion can be ignored as similar husbandry practices were adopted in both trials for all 3 treatments. These findings are in line with the work of Dharma et al., (2014), who have blamed AI for its role in deterioration of chick quality. The comparison of treatments of AI in both trials directed that it would have been facilitating the proliferation and intensification of pathogens such as *E. coli* and SP. Whereas, these both bacteria replicate swiftly in the intestine of newly hatched chicks, and infection spreads rapidly from chick to chick, resulting into higher early chick mortality. Some other studies also refer the higher risk of infection owing to mild careless handling of AI resulting in chicks affected with single or multiple infections (Corrier et al., 1999; Buhr et al., 2005; Kabir, 2010). However, it is deducted from both trials that chicks of caged flock have better livability and lesser depletion instead of facing AI as compared with progeny of floored flock but lesser than of NM.

Table 4. Effect of different mating strategies on progeny growth performance.

Treatment	Body weight (g)			Feed intake (g)			FCR			EEF		
	Peak	Postpeak	*P*-value	Peak	Postpeak	*P*-value	Peak	Postpeak	*P*-value	Peak	Postpeak	*P*-value
AIF	2,117.00 ± 5.99^{b}	2,147.00 ± 6.45^{b}	0.42	3,241.00 ± 2.99^{y}	3,470.00 ± 2.99^{x}	<0.0001	1.56 ± 0.006^{a,y}	1.64 ± 0.005^{a,x}	0.000	360.11 ± 3.23^{b,x}	334.95 ± 4.42^{b,y}	<0.0001
AIC	2,105.67 ± 6.93^{b}	2,117.00 ± 6.92^{b}	0.42	3,215.00 ± 4.05^{y}	3,468.00 ± 4.02^{x}	<0.0001	1.56 ± 0.004^{a,y}	1.63 ± 0.006^{a,x}	0.000	362.96 ± 3.48^{b,x}	342.5 ± 4.23^{b,y}	<0.0001
NM	2,216.23 ± 10.83^{a}	2,225.00 ± 12.11^{a}	0.36	3,257.00 ± 2.95^{y}	3,471.00 ± 3.15^{x}	<0.0001	1.49 ± 0.008^{b,y}	1.56 ± 0.008^{b,x}	0.000	404.8 ± 5.0^{a,x}	386.54 ± 4.89^{a,y}	<0.0001
P-value	<0.0001	<0.0001		0.0671	0.0825		<0.0001	<0.0001		<0.0001	<0.0001	

$^{a\text{-}b}$Superscripts on different means within column differ significantly among different ages at $P \leq 0.05$.
$^{x\text{-}y}$Superscripts on different means within row differ significantly among different mating strategies at $P \leq 0.05$.
Abbreviations: AIF, artificial insemination in floor flock; AIC, artificial insemination in caged flock; EEF, European efficiency factor; FCR, feed conversion ratio; NM, natural mating in floor flock.

Table 5. Effect of different mating strategies on progeny immune response.

		Peak				Postpeak			
	Day	AIF	AIC	NM	*P*-value	AIF	AIC	NM	*P*-value
H9	0	2.94 ± 0.37	2.67 ± 0.42	3.14 ± 0.40	0.70	2.69 ± 0.69	2.80 ± 0.75	2.90 ± 0.25	0.63
	15	1.69 ± 0.69	1.55 ± 0.74	1.22 ± 0.63	0.96	1.32 ± 0.87	1.40 ± 0.45	1.37 ± 0.39	0.84
	30	1.52 ± 0.92	1.94 ± 0.37	1.55 ± 0.53	0.40	1.15 ± 0.93	1.29 ± 0.25	1.55 ± 0.53	0.32
P-value		0.083	0.068	0.058		0.081	0.080	0.082	
ND	0	5.00 ± 1.00^{c}	4.33 ± 0.80^{c}	4.67 ± 1.61^{c}	0.40	3.90 ± 1.05^{c}	4.19 ± 0.66^{c}	4.27 ± 1.75^{c}	0.40
	15	13.33 ± 3.96^{b}	11.69 ± 2.38^{b}	12.87 ± 2.29^{b}	0.92	15.33 ± 1.77^{b}	13.44 ± 2.57^{b}	11.55 ± 1.88^{b}	0.84
	30	44.69 ± 10.89^{a}	53.48 ± 15.98^{a}	50.75 ± 22.46^{a}	0.32	36.92 ± 11.2^{a}	38.99 ± 20.77^{a}	44.58 ± 18.66^{a}	0.38
P-value		<0.0001	<0.0001	<0.0001		<0.0001	<0.0001	<0.0001	
IB	0	103.67 ± 26.59^{c}	106.67 ± 42.75^{c}	115.50 ± 69.04^{c}	0.63	98.878 ± 20.13^{c}	93.33 ± 50.75^{c}	117.90 ± 77.45^{c}	0.73
	15	1,744.67 ± 54.92^{b}	1,785.33 ± 54.69^{b}	1,856.97 ± 76.56^{b}	0.23	1,767.99 ± 40.89^{b,y}	1,517.66 ± 49.65^{b,z}	1,846.33 ± 78.16^{b,x}	0.03
	30	3,193.33 ± 161.37^{a}	3,244.50 ± 199.69^{a}	3,323.83 ± 189.23^{a}	0.98	3,088.59 ± 126.65^{a}	3,150.60 ± 175.5^{a}	3,286.58 ± 143.83^{a}	0.81
P-value		0.0001	0.0001	0.0001		0.0001	0.0001	0.0001	

[a-c]Superscripts on different means within column differ significantly among different days at $P \leq 0.05$.
[x-z]Superscripts on different means within row differ significantly among different mating strategies at $P \leq 0.05$.
Abbreviations: AIF, artificial insemination in floor flock; AIC, artificial insemination in caged flock; H-9, Avian Influenza; IB, infectious bronchitis; NM, natural mating in floor flock; ND, New Castle Disease.

Growth Performance

Growth performance was found significantly ($P \leq 0.05$) different among all treatments in both trials (Table 4). According to results, experimental birds produced through NM exhibited significantly better body weight gain and FCR when being grown with experimental birds produced through AI in caged and floored flock, respectively. As the better FCR along with body weight gain and livability % (Table 3) were recorded by the chicks of NM, they presented the best EEF followed by chicks of AI in caged and floored flocks, respectively (Table 4). Data of livability % have already been mentioned in Table 3, and it was the best in experimental birds of NM than of AI in caged and floored PF. Whereas, the results of second trial legitimated the findings of first trial and gravity of differences in growth parameters (body weight gain, FCR, EEF) among the treatments that became more obvious ($P \leq 0.05$) (Table 4). On comparison of trials 1and 2, chicks from peak phase production performed better as compared with chicks of older PF under all 3 treatments. However, feed intake remained nonsignificant in all 3 treatments in both trials. Growth performance is considered a fundamental parameter in most of production studies that is governed by multiple factors. Microclimatic conditions, pathogenic load, and handling of equipment can be influential factors to affect the ultimate growth performance. Depression in growth of AI chicks can be directly associated with the chick quality, and during AI, the chick quality would have compromised because of high pathogenic load, particularly of *E. coli*, SP, and MG, which might have exerted negative impact on growth performance. Therefore, it is imperative that AI might have retro impact on hygienic status of embryo which kept the birds under stress particularly in early life in comparison to NM (Willemsen et al., 2008; Iqbal et al., 2017). It seems that method of insemination would have indirectly regulated the growth performance by meddling with chick's hygiene (Li et al., 2018). As chicks representing AI possessed higher contamination of *E. coli*, Salmonella, and MG (Table 1), it led to higher mortality in chicks of AI resulting into poor FCR and livability%. These both factors dented adversely to EEF by the chicks of AI. It can be concluded that hygienically compromised chicks produced through AI would have died in early life of posthatch, and stress would have be existed in whole life in general, as process of AI was carried out in the shed where the microclimate might be enriched with many a pathogen. Such pathogens might have incorporated at any stage and spread to chick ultimately. Such chicks could not show improvement growth performance even eating almost same quantity of feed. The principal cause of poor growth performance shown by from AI chicks was poor livability, which regulated the FCR and EEF. Omphalitis mainly caused by *E. coli* and salmonella led to higher mortality in early posthatch, whereas MG would have hampered the growth in later stage of life of broiler originated by AI in caged and floored flocks (CPRC, 2018).

Immune Response

Immune response in pursuance of live vaccines of ND and IB was recorded slightly better in experimental birds obtained through NM as compared with chicks of AI in both trials (Table 5). The results of sampling conducted at 0 D in both trials revealed that maternal antibodies that level against Avian influenza H-9, IB, and ND were found nonsignificantly higher in DOC of NM as compared with progeny of AI. Significant increasing trend in titers of ND and IB was recorded with progression of age of all experimental birds in both trials (Table 5). Although, AI in PF might have exerted inert influence in responding to vaccines in broiler directly, yet it would have mitigated the immune capacity of birds by inducing or provoking bacterial infection. Therefore, slightly better response of progeny of NM to vaccines of ND and IB might be because of their better hygienic status along with better body weight. However, difference in maternal antibodies level of above-mentioned diseases reflected some meddling role of insemination method on immune status of PF which was being expressed in DOC (Table 5). Therefore, infected chicks (Table 1) would have remained immune compromised; therefore, vaccines could not instigate the defensive mechanism properly (Hoerr, 2010). Results of this study resembled with findings of Badowski et al. (2014) and Schultz et al. (2019), and there are a couple of intrinsic and extrinsic factors which obsessed the immune compromise phenomenon including mycotoxin and bacterial infection, particularly infection of *E. coli*, SP, and MG. Whereas, it can be drawn from results that any

Table 6. Effect of different mating strategies on progeny slaughtering parameters.

Treatment	AIF	AIC	NM	*P*-value
Live Weight (g)	2,198.00 ± 106.48	2,324.67 ± 115.31	2,335.33 ± 101.98	0.31
Carcass Weight (g/100 g)	64.29 ± 1.53	67.81 ± 2.23	67.86 ± 2.10	0.41
Breast Weight (g/100 g)	27.29 ± 1.52	31.11 ± 1.62	31.07 ± 3.22	0.41
Thigh Weight (g/100 g)	15.13 ± 0.42	12.52 ± 0.75	14.02 ± 0.79	0.08
Heart Weight(g/100 g)	0.46 ± 0.05	0.52 ± 0.03	0.49 ± 0.53	0.73
Spleen Weight (g/100 g)	0.11 ± 0.007	0.12 ± 0.03	0.11 ± 0.01	0.94
Gizzard Weight (g/100 g)	2.98 ± 0.15	2.74 ± 0.13	2.64 ± 0.28	0.51
Liver Weight (g/100 g)	2.77 ± 0.04	2.80 ± 0.10	2.49 ± 0.14	0.15
Intestine Weight (g/100 g)	4.43 ± 0.57	4.93 ± 0.05	3.88 ± 0.31	0.23

Abbreviations: AIF, artificial insemination in floor flock; AIC, artificial insemination in caged flock; NM, natural mating in floor flock.

kind of stress on PF might be reflected in maternal antibodies in progeny, and such narrative also was earlier studied by some scientists such as Christina et al. (2014).

Slaughtering Parameters

Nonsignificant difference in carcass characteristics was recorded, which included weight of carcass yield (g), breast, thigh, as well as weight of internal organs (heart, spleen, gizzard, liver, and intestine) (g/100 g), when birds of almost similar body weight were slaughtered at end of both trials (Table 6). Five birds of almost same body weight were selected from all replicates of 3 treatments to avoid the influence of different final body weight on mentioned parameters as purpose was rather just to monitor the impact of insemination methods (AI and NM). In lieu of such slaughtering, it was perceived that neither AI nor NM exerted any effect on carcass characteristics, but there is a direct correlation of final body weight to the above said carcass characteristics as found by some researchers such as Pundir et al., 2011. But, husbandry practices along with birds hygiene can regulate the poultry processing (Eduardo, 2015; da Silva et al., 2017). Whereas, it was noticed in this study that birds produced through AI manifested lesser body weight (Table 3); as a result, birds could exhibit lesser readings of abovementioned parameters in general. Such findings that "heavier birds manifested better carcass characteristics" are independent to way by which they are being produced, and it is in accordance to the findings of Udeh and Obgu (2011) who found that organ weight is in proportionate to its body size and weight or depend on its nutrition (Fernando, 2015; Li et al., 2016).

CONCLUSIONS

It is concluded from the results of both trials that the best growth performance (EEF, FCR, WG, L%) along with the least depletion and microbial load of concerned pathogens (*E. coli*, Salmonella pullorum, Mycoplasma gallicepticum) were pertained by the experimental birds representing NM as compared with chicks being produced through AI in floored and caged flock, respectively. While, experimental birds being produced through AI in floored flock particularly during postpeak phase exhibited the worst growth performance and the highest contamination of concerned microbiota and mortality % in first 3 wk of age. However, this phenomenon needs to be probed more.

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