



## MATERIALS AND METHODS

### Ethical approval

This experiment was a routine field work in hatchery considering all rules and regulations regarding animal rights and ethic, university of veterinary and animal sciences, Lahore, Pakistan.

### Site selection

This study was carried out at Sadiq Poultry (Pvt) Limited, Chakri Hatchery Rawalpindi which is situated 5 km from chakri interchange on motorway (M2). The hatchery contains the latest Heating Ventilation and Air Conditioning (HVAC) automation, having ISO (International standard organization) 1900-2000 certified. This hatchery has the largest eggs capacity in south of Asia, which is producing the best quality of chicks through single stage incubation system (Avida G4, Chick Master USA).

### Egg selection

High-quality hatching egg with good quality shells, without ridges or small lumps of calcified material (pimples) were selected. The eggs were graded on the basis of their quality and weight, all the hatch able eggs were graded through egg grading machine MOBA 9A. While the poor shell, crack, bloody stained, elongated eggs were rejected (Khan et al., 2016), only oval shape and good quality eggs were selected. Egg room temperature and humidity were kept at 75 °F and 65 respectively with fresh air 2 CFM/1000 eggs during the course of the study.

### Group/Breed classification

Broiler breeders were classified into three groups young (age breeder eggs 24-31 weeks), prime (age breeder eggs 32-50 weeks), and old (age breeder's eggs 50+ weeks) according to breeder's age. Each experimental group was consisted of (n=8640, 000) eggs with sixteen replicates for each group (n=540,000) eggs.

### Selected Flocks

The young group contains Sadiq Poultry (SP) flock no: 102-1 ross, 105 ross, 106-1 hubbard classic, 106-2 and Arsaln Poultry (AP) flock no: 22-1 ross, 22-2 ross. Prime group contains SP flock no: 103 Cobb, 94 cobb, 98ross, 99ross, AP flock no: 21-cobb-1, 21-cobb-2, Khan Poultry (KP-6 Arbor Acre) flock no: 6, Rahim Farm (RF-9 Cobb) flock no: 9. Old group contains SP flock no: 90-cobb-2 , 90-hubbard classic -2, 92-1 hubbard classic, 92-2 hubbard classic, 96 cobb, AP flock no: 22 Ross, Kaloo Farm (KRA, KRB) cobb, Attock Farm (ATK Ross), rose (RAC Ross) and Green land (GI-Cobb-3).

### Egg weight

Before setting the eggs weight of each individual group was calculated by the formula:

$$\text{Egg weight: } \frac{\text{Full tray weight at Setting- Weight of empty tray}}{\text{Total No of eggs in tray}}$$

### Egg fumigation

Before the weighing, the trial eggs were fumigated with 20 g KMnO<sub>4</sub> and 40ml formalin (40%) and 40 ml of water for 100ft<sup>3</sup> areas for 15 minutes through automatic fumigation process provided by Chick Master.

### Incubation programme

All the groups had been pre-heated at 82°F for 5 hours inside incubators. After completion of the pre-warming the setter started automatically the incubation stage profile (Recommended by Chicks Master USA). Incubation duration for young, prime and old was 456 hours in setter (19th day) and 50 hours in hatchers.

### Setter hall and hatcher hall

Environmental conditions in setter hall were at 75 °F temperatures and 40% Relative humidity; whereas in the hatcher hall temperature was at 75 °F and relative humidity had been increased up to 60%. The positive pressure in setter and hatcher hall was 15 Pascal and 10 Pascal respectively, while negative pressure inside setter and hatcher plenum was - 25 Pascal during the course study.

### Candling

Fertility of eggs were performed through candling then shifted to hatchers for next 50 hrs. These entire incubation stage programs have been recommended by chick master USA.

### Egg's water loss

Before being transferred to hatcher's water loss was measured from each group individually after 456hrs of incubation. Water loss was measured by given formula:

$$\text{Water Loss \%} = \frac{\text{Full tray weight at Setting} - \text{Full Tray Weight at Transfer}}{\text{Full tray weight at Setting} - \text{Empty Tray Weight}} \times 100$$

### Chick yield measure

After hatch out immediately, the chick's weight and yield were measured through electrical weight balance by using following formula:

$$\text{Chick Yield \%} = \frac{\text{Weight of chicks} \times 100}{\text{Egg weight}}$$

### Hatch window

Hatch window is the duration between the 1<sup>st</sup> chick hatches out up to last chick hatch out (Noiva et al., 2014). Range of hatch window is 20-24 hours. For prime and old it was 22-24 hours while 20-22 hours for young.

### Chick grading

Grading of chicks was performed on conveyor, automatic grading table. Only stranded (shining eyes, soft legs and nose, healed naval and healthy chicks) were shifted to chick's box after counting, while under weight, weak, and unhealed naval chicks were removed as international standard as describe by (Yousaf et al., 2017).

### Dead in shell (DIS) analysis

To investigate the reason of embryo's mortality inside the eggs, un hatched eggs were broken. For this purpose analysis of un-hatch eggs was performed as presented in table 2 and their details are presented in table 3, 4 and 5.

### Statistical analyses

All data were analyzed by using Statistical Analysis System package software (SAS version 9.2, SAS Institute Inc., Cary, NC, USA). All means were compared using Duncan's Multiple Range test and results were presented as mean  $\pm$  SEM (standard error of mean). Results were considered significant if  $P < 0.05$ .

## RESULTS

Hatchability ( $81.98 \pm 1.1^a$ ,  $88.44 \pm 1.6^b$ ,  $79.60 \pm 1.6^c$ ), candling ( $10.25 \pm 1.25^a$ ,  $6.44 \pm 0.88^b$ ,  $10.73 \pm 1.25^a$ ), DIS ( $7.7 \pm 0.4^a$ ,  $5.1 \pm 0.6^b$ ,  $9.6 \pm 0.6^c$ ), water loss ( $11.29 \pm 0.11^a$ ,  $11.77 \pm 0.16^b$ ,  $12.13 \pm 0.16^c$ ), egg weight ( $57.2 \pm 0.5^a$ ,  $64.2 \pm 0.7^b$ ,  $70.5 \pm 0.7^c$ ), Chick weight ( $39.4 \pm 0.3^a$ ,  $43.9 \pm 0.5^b$ ,  $48.15 \pm 0.5^c$ ) were significantly ( $P < 0.001$ ) different for young, prime and old respectively. For crack ( $0.5 \pm 0.12^a$ ,  $0.5 \pm 0.17^a$ ,  $1.1 \pm 0.17^b$ ) and contaminated eggs ( $0.63 \pm 0.09^a$ ,  $0.50 \pm 0.12^a$ ,  $1.31 \pm 0.12^b$ ) young and prime were significantly ( $P < 0.0001$ ) better than old as shown in table 1. The high percentage of crack eggs in older flocks is due to thin egg shell. Young, prime and old were significantly different for early embryonic mortality ( $3.07 \pm 0.3^a$ ,  $3.46 \pm 0.2^b$ ,  $4.55 \pm 0.3^c$ ) respectively, while for mid ( $1.04 \pm 0.13^a$ ,  $0.53 \pm 0.09^b$ ,  $1.0 \pm 0.13^a$ ) and late ( $3.77 \pm 0.4^a$ ,  $2.58 \pm 0.2^b$ ,  $3.28 \pm 0.4^a$ ) prime was significantly better than young and old as shown in table 3. Mal-Position and Mal-Formation were at 1.5% and 0.5% for total eggs set respectively as shown in tables 4 and 5. The age of breeders does not affect significantly the chick yield, chick waste, Mal-position and Mal-formation. Mal-position and Mal-formation are mostly related to flock health condition, hatchery management and some genetic disorder regardless of the age of breed. Prime flock was found to have better result in term of hatch ability, candling, DIS, contamination of eggs and crack eggs percentage.

**Table 1.** Effect of broiler breeder's age on eggs weight, crack eggs, contaminated eggs, chick weight, water loss, and chicks yield and egg waste in Sadiq hatchery Pakistan at March 2016

Parameters	Group A (Young)	Group B (Prime)	Group C (Old)
Incubation durations (h)	$506.2 \pm 0.4^a$	$506.2 \pm 0.4^a$	$506.2 \pm 0.4^a$
Egg Weight (g) Day 1 <sup>st</sup>	$57.2 \pm 0.5^a$	$64.2 \pm 0.7^b$	$70.5 \pm 0.7^c$
Crack eggs (%)	$0.5 \pm 0.12^a$	$0.5 \pm 0.17^a$	$1.1 \pm 0.17^b$
Contaminated eggs (%)	$0.63 \pm 0.09^a$	$0.50 \pm 0.12^a$	$1.31 \pm 0.12^b$
Chicks Weight (g)	$39.4 \pm 0.3^a$	$43.9 \pm 0.5^b$	$48.15 \pm 0.5^c$
Water Loss (%)	$11.29 \pm 0.11^a$	$11.77 \pm 0.16^b$	$12.13 \pm 0.16^c$
Chick Yield (%)	$68.99 \pm 0.2^a$	$68.46 \pm 0.4^a$	$68.72 \pm 0.4^a$
Egg Waste (%)	$19.71 \pm 0.3^a$	$19.75 \pm 0.4^a$	$19.13 \pm 0.4^a$

**Table 2.** Effect of broiler breeder's age on hatchability, candling and dead in shell in Sadiq hatchery Pakistan at March 2016

Parameters	Group A (Young)	Group B (Prime)	Group C (Old)
Hatchability (%)	81.98±1.1 <sup>a</sup>	88.44±1.6 <sup>b</sup>	79.60±1.6 <sup>c</sup>
Candling (%)	10.25±1.25 <sup>a</sup>	6.44±0.88 <sup>b</sup>	10.73±1.25 <sup>a</sup>
DIS (%)	7.7 ±0.4 <sup>a</sup>	5.1 ±0.6 <sup>b</sup>	9.6±0.6 <sup>c</sup>

**Table 3.** Effect of broiler breeder's age on early, mid and late embryonic mortality in Sadiq hatchery Pakistan at March 2016

Parameters	Group A (Young)	Group B (Prime)	Group C (Old)
Early embryo dead (0-7 da) (%)	3.07±0.3 <sup>a</sup>	3.46±0.2 <sup>b</sup>	4.55±0.3 <sup>c</sup>
Mild embryo dead (8-14 day) (%)	1.04 ±0.13 <sup>a</sup>	0.53 ±0.09 <sup>b</sup>	1.0±0.13 <sup>a</sup>
Late embryo dead (14-21 day) (%)	3.77 ±0.4 <sup>a</sup>	2.58 ±0.2 <sup>b</sup>	3.28±0.4 <sup>a</sup>

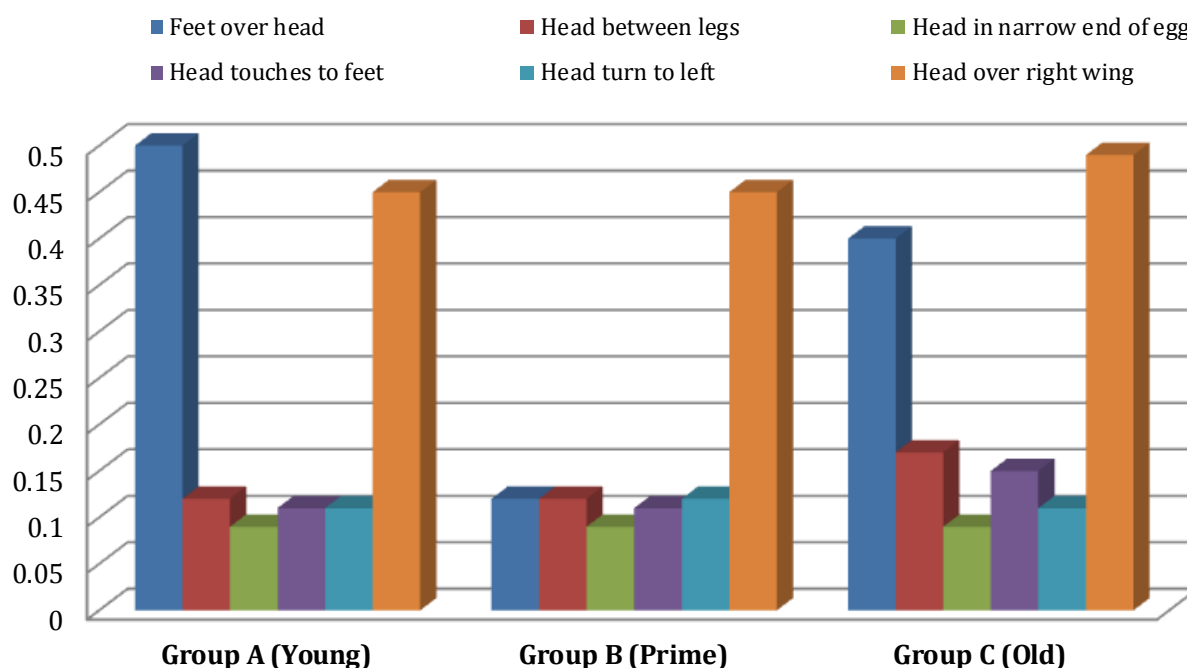
## DISCUSSION

Results of sixteen successful hatches had been recorded for each individual group. Hatchability, candling, DIS Chick yield, water loss and chick weight were recorded for every group individually (Tables 1 and 2). For all three groups incubation duration was insignificant ( $P>0.05$ ) 506 hrs table 1. Incubation duration of 506 hours is good for chicks in term of chick's quality, yield, water loss (Jabbar et al., 2017). The young flock which may hatch in (502-504 hours) while for old it may goes to (506-510 hours) (King'ori, 2011). Hatch window is directly related to pre-warming, incubation temperature and breeders age for good quality chicks (Yousaf et al., 2017). Fresh eggs were weighted (g) before being set to incubation. Fresh eggs were significantly different ( $P<0.001$ ) for young, prime and old. Weight of young age breeder's eggs was less in weight due to small size while egg weight of prime and old age breeders was better due to age difference Table 1. The standard weight and size of egg are recommended for incubation according to breeders age but it may be affected due to multiple reasons, such as age and breed type, diseases in breeder (ND, IB, H9, EDS, MG etc.), and farm management issue (King'ori, 2011). Crack eggs depend on egg shell quality and breeders age. The crack eggs percentage is significantly better ( $P<0.001$ ) in young and prime as compare to old Table 1. Egg's shell of young and prime breeder are thick and hard that is why crack percentage has not been recorded in such flocks, while it is recorded to the maximum in old flock (Khan et al., 2016) Table 1. The contamination significantly ( $P<0.001$ ) increases from young to prime then old. The contaminated eggs also depend upon flock health status and farm management as shown in table 1 (Reis et al., 1997). The weight of chicken significantly increased ( $P<0.001$ ) from young to prime then old as shown in table 1. Chick weight is affected by multiple reasons, such as ideal water loss, contamination free eggs, ideal incubation duration (506 hours), short hatch window and ideal temperature and humidity in setter/Hatcher and chick hall (Jabbar et al., 2017). Water loss had significantly increases from young to prime then old as shown in table 1. Water loss from hatch able eggs during incubation is critical for good chick yield and chick weight. To ensure the good quality of chicks ideal water loss and chick yield are the key factor (Yousaf et al., 2017).

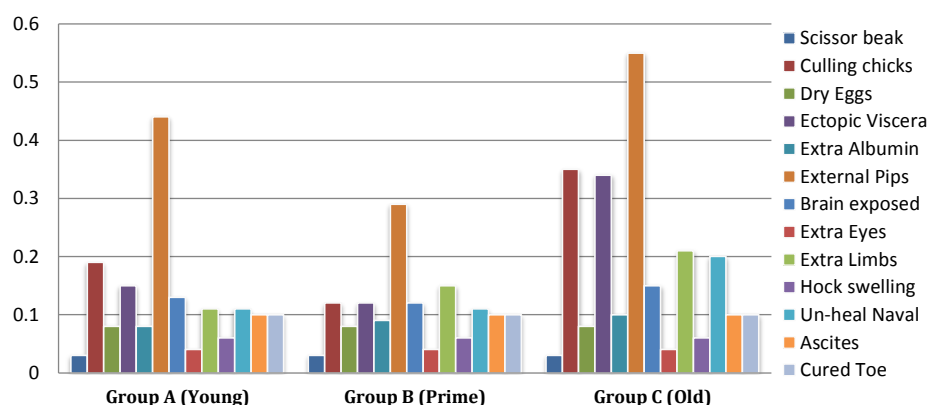
The chick yield is percentage of chick conversion from egg. Chick yield is non-significantly ( $P>0.001$ ) for young, prime and old Table 1. Water loss was significantly different among breeder flock (young, prime, old), where as the chick yield will be remain same which will be not affected by age of flock (Tong et al., 2013). The percentage of chick conversion from egg remains the same in young, prime and old. Eggs waste was significantly ( $P<0.001$ ) similar in percentage, but it be different by weight table 1. The older flocks will contain high waste as compare to young or prime (Løtvedt and Jensen, 2014). Hatchability, candling and DIS were significant ( $P<0.001$ ) in young to prime and old as shown in table 2. The young flock's fertility depends upon the time of male being introduced to the flock (Van de Ven, 2012), whereas prime flock goes peak hatchability (Yousaf, 2016) and then for older flocks there is decreasing trend for hatchability (Van de Ven, 2012). The decline in reproductive performance is well documented after 45 weeks of age (Van de Ven, 2012). The age of breeder flock had affected the reproductive performance (Yousaf, 2016). Candling of breeders flock ( $P<0.001$ ) had increased significantly in young to prime and old respectively as shown in table 2. The candling, hatchability and DIS are related to each other. There are so many factors which affect the hatchability of flock such as health status of flock, diseases issues, spiking, induce molting and farm management (Jabbar et al., 2015). These factors are responsible for change in Hatchability, candling and DIS regardless the age of breeders (Vieira et al, 2005). DIS (dead in shell) ( $P<0.001$ ) had significantly increased in young, prime and old respectively as shown in table 2. DIS

analysis is good tool to assess hatchery performance. The early, mid and late embryonic mortality significantly ( $P < 0.001$ ) increase according to age of breeders as shown in table 3. The early embryonic mortality had also been affected by temperature shock, pre-warming, condensation, transportation condition, egg room temperature, storage duration and condition at the farm, fumigation, ventilation, turning and initial (0-7 days) temperature and humidity set points (Van de Ven et al., 2011). The mid (7-14 days) embryonic mortality occurs due to improper incubation condition in setters including temperature and humidity, turning, ventilation, over or under heat. The reason for late (15-21 days) embryonic mortality is improper incubation condition in setters and mostly in hatchers such as high temperature, low humidity, ventilation, turning, jerk during transfer, inadequate transfer temperature, improper hatch window and incubation duration (Løtvedt and Jensen, 2014).

The embryonic mortality at any stage early, mid and later may also be affected by breeder health status and vertically transmitted diseases such as salmonella, MG and Adeno Virus (Vieira et al., 2005). Range of hatch window was 20-24 hours. For prime and old it was 22-24 hours while for young 20-22 hours. Older flock eggs required more heat in the early stage and produced more heat as compared to young flock eggs in later stages, which increases their hatch window. Mal-positions such as feet over head, head between legs, head touching to feet, head turn to left and head over right wing all these conditions are due to improper turning and low humidity, disease and vitamin deficiency specially Vitamin B complex (Tong et al., 2013). Head in narrow end of egg is due to eggs blend end were placed up during egg setting. All these mal-positions were non-significant for young, prime and old as shown in graph 1. Such kind of mal-positions should remain at 1.5% of total eggs set regardless the age of breeders (Tong et al., 2013). Expose brain, ectopic viscera, extra limbs, unhealed navals, excessive albumin, Ascites, Hock swelling, extra eyes, scissor beak, curved toe, dry eggs contents and culling are common mal-formation that occur during incubation as shown in graph 2 (Yildirim et al., 2004). The malformations were insignificant ( $P > 0.001$ ) according to the age of breeders as shown in graph 2. The reason for exposed brain, excessive albumin, Ascites, ectopic viscera and extra limbs is mostly due to high incubation conditions (Yildirim et al., 2004). Hock swelling occurs due to hard egg shell of young and prime age breeder egg or it may be due to long time incubation in plastic Hatcher trays (Tong et al., 2013). Extra eyes, scissor beak and curved toe may be due to high incubation conditions and genetical disorders (Tong et al., 2013). Dry egg content is due to hairline crack due to which the egg content becomes dry. Un-heal naval are very common problem occurring due to being under heat high humidity or contamination inside machines. This condition may also develop due to vertical diseases (Vieira et al., 2005). Culling rate again depends upon the health condition of flock and incubation conditions of machines. The culling rate had increased in young, prime and old respectively. All these malformation should be less than 0.5% of total eggs set for a good hatch as shown in graph 2 (Tong et al., 2013).



**Graph 1.** Late embryo dead (14-21 days) due to Mal- Position of embryo



**Graph 2.** Late embryo dead (14-21 days) due to Mal-Formations of embryo

## CONCLUSION

The age of broiler breeders affects the quality investigation factors of hatchery i-e hatchability, candling, DIS, water loss, egg weight, chick yield and Chick weight. The age of breeds does not affect Mal-position and Mal-formation. Prime flock was found to have better results in term of hatchability, candling, DIS, contamination of eggs and crack eggs percentage.

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### Author's contribution

Both authors have equally contribution in this work.

### Competing of interest

The authors declare that they have no conflict of interest with respect to the research, authorship, and/or publications of this article.

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