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Article

Performances and adaptability of two upgraded dwarf layers under smallholder farmers' condition

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Abstract: Upgrading of indigenous chicken with exotic breed is an important way to improve their productivity for the traits of economic interest. The experiment was conducted to evaluate the productivity, profitability and adaptability of two up-graded dwarf chicken populations under smallholder farmers' condition. A total of 72 birds of third generation (F₃) of Dwarf-Fayoumi (IDC \circlearrowleft × Fay \circlearrowleft) and Dwarf-Leghorn (IDC \circlearrowleft × WLH \circlearrowleft) at 12 weeks of age were distributed to 12 farmers nearby three villages (Kewatkhali, Boyra, Sutiakhali) of Bangladesh Agricultural University, Mymensingh. Birds were reared at farmers' house for a period of 16 weeks with same feeding and management practices. Farmers were grouped into two (6 farmers in each group) for allocating IDC $\lozenge \times \text{Fay}$ and IDC $\lozenge \times \text{WLH}$ upgraded genotypes. Each farmer was given six birds of same genotype. The study revealed that IDC $\circlearrowleft \times$ Fay \circlearrowleft pullets showed earlier sexual maturity (147.50±0.76 days) and lower feed intake (74.55±0.23 g/bird/day) and thus had a better FCR (2.98±0.02) during the experimental period. The corresponding values were found 169.83±1.35 days, 76.07±0.19 g/bird/day and 3.51±0.02, in IDC × WLH^Q genotype. Moreover, among the production traits considered egg mass, hen day egg production, performance efficiency index and egg feed price ratio were found higher among the IDC $\partial \times$ Fay \bigcirc pullets as 25.07 \pm 0.20 g/bird/day, 37.48 \pm 0.43%, 21.22 \pm 0.95, and 1.175 \pm 0.015, respectively. The survivability of IDC \Diamond × Fay \bigcirc and IDC \bigcirc × WLH \bigcirc upgraded genotypes were 94.4 and 77.7%, respectively during the experimental period. Therefore, it can be concluded that the IDC $\lozenge \times \text{Fay} \supseteq \text{pullets}$ were found more productive, profitable and had better adaptability than IDC♂ × WLH♀ pullets under smallholder farmers' management. The resultant $IDC_{\circlearrowleft}^{\land} \times Fay_{\downarrow}^{\circlearrowleft}$ upgraded chicken could be utilized as mini layer under rural settings of Bangladesh.

Keywords: upgradation; production; performance efficiency index; chicken; Bangladesh

1. Introduction

Crossbreeding and upgrading of indigenous chicken was considered the simplest and quickest way to improve indigenous stock for economically significant traits (Padhi, 2016; Mengesha et al., 2022). By introducing

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upgraded or crossbred genotypes, the egg production at smallholder level might be increased within the existing production system in Bangladesh (Khan *et al.*, 2006; Islam *et al.*, 2015). After 1990, Sonali (RIR \circlearrowleft × Fayoumi \updownarrow) was introduced through Department of Livestock Services (DLS) to increase village level egg and meat production (Ali *et al.*, 2022). The majority of previous efforts were centered on enhancing native fowl through upgrading (Faruque *et al.*, 2015). However, little attention has been paid to the adaptability of crossbred or upgraded chickens in hot-humid country like Bangladesh. In addition, poor management and fluctuating supply of feed are major concerns for optimum productivity under smallholder farming condition (Wong *et al.*, 2017; Wilson *et al.*, 2022). Therefore, Bangladesh needs a type of chicken having an intermediate egg production between poor producer native and high yielding commercial strain with lower maintenance and higher adaptability at household level (Chowdhury, 2013).

In today's context, global warming or climate change poses a significant concern, as higher ambient temperatures necessitate increased energy (feed) requirements for chickens compared to thermoneutral conditions (Kumar *et al.*, 2021). Poor feed conversion has a significant negative impact on the production and health of chickens, leading to major losses (Anene *et al.*, 2021). The dwarf chickens would be more resistant to the effect of high temperature and relative humidity (Nawaz *et al.*, 2023). The dwarf gene's reduction in body size plays a significant role in the acclimatization of chickens to the tropics through radiation and convection (Fathi *et al.*, 2022). The dwarf chicken has an extra genetic advantage that allows it to retain more water in its body, making it more heat resistant (Onagbesan *et al.*, 2023; Nawaz *et al.*, 2024). Heat stress has less depressive effect on egg production in dwarf hens than the normal birds (Oluwagbenga and Fraley, 2023). Better productivity and survivability of dwarf chickens under heat stress are linked to relatively reduced body sizes and increased sodium contents in their plasma (Wasti *et al.*, 2020; Onagbesan *et al.*, 2023). Thus, dwarf chickens require less care and nutrition than their normal-sized counterparts, are more resistant to heat and disease, and produce more eggs (Yeasmin and Howlider, 1998; Manyelo *et al.*, 2020).

The following benefits of mini hens are the reason for the current interest in the practical application of dwarf gene: superior utilization of feeds for production, higher survivability, higher stocking density, superior reproduction capability and increased heat stress resistance (Decuypere *et al.*, 2010; Mincheva *et al.*, 2015; Ibrahim *et al.*, 2019; Manyelo *et al.*, 2020; Mangan and Siwek, 2023). Therefore, it might be possible to meet demand and increase the production of chicken meat and eggs at the village level by introducing dwarf types and synthesizing them using native autosomal dwarf fowls and exotic breeds. This would also help to reduce adult body size, improve feed efficiency, and increase adaptability in a hot-humid tropical climate. So, the productivity of chicken raised in rural condition would improve with the production of upgraded chickens using exotic breed (White Leghorn and Fayoumi) with indigenous dwarf chickens.

Earlier studies reported the performances of indigenous dwarf chicken derived upgraded genotypes mostly under intensive management condition (Yeasmin and Howlider, 1998; Ferdaus *et al.*, 2016; Faruque *et al.*, 2017). Importantly, productivity and adaptability of crossbred or upgrade genotypes differ largely under farmers' condition due to interactions between genotype and environment. However, until to date, there is lack of information on performance and adaptability of upgraded or crossbred chicken under rural settings and therefore, is essential to validate their genetic potentiality and adaptive capacity under the aforesaid production environment. Under the circumstances stated above, the current study was designed to compare the productivity, profitability and adaptability of two up-graded dwarf chicken under smallholder farmers' condition.

2. Materials and Methods

2.1. Ethical approval

No ethical approval was required to conduct this study as birds were not sacrificed or injured for this experimentation.

2.2. Experimental chickens and farmers

A total of 72 upgraded birds of third generation (F3) of Dwarf-Fayoumi (IDC \circlearrowleft × Fayoumi \circlearrowleft) and Dwarf-Leghorn (IDC \circlearrowleft × WLH \circlearrowleft) pullets at 12 weeks of age were selected to conduct this experiment. Three villages adjacent to Bangladesh Agricultural University, Mymensingh (Kewatkhali, Boyra, Sutiakhali) were selected for the adaptability trial. The villages were located at coordinates 24°40′0″N latitude and 90°26′40″E longitude (Figure 1).

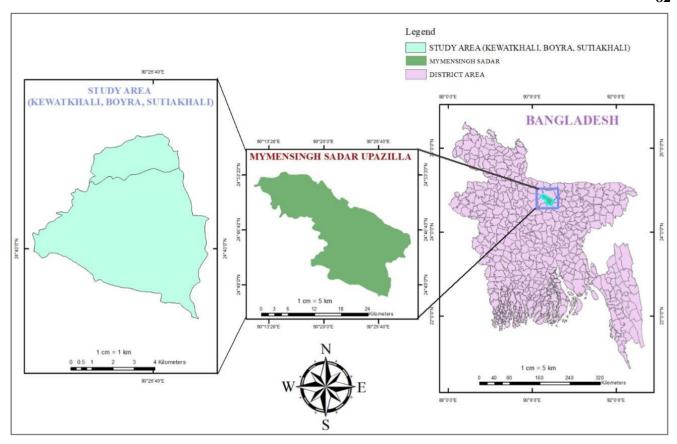


Figure 1. Map of study area near Bangladesh Agricultural University, Mymensingh.

A total of 12 farmers were selected taking four from each village and were grouped into two having 6 farmers in each group. The IDC \circlearrowleft × Fayoumi \circlearrowleft upgraded birds were distributed to first group and the farmers belong to second group reared IDC \circlearrowleft × WLH \circlearrowleft birds. Each farmer was given six birds of same genotype. The layout of the experiment is shown in Table 1. Birds were reared under the farmers' house for a period of 16 weeks (up to 28 weeks of age) from July 2016 to October 2016. The farmers were provided a guideline to fulfill the objectives of the research.

Table 1. Layout of experimental design of birds.

Villages	No. of Pullets				
	Dwarf-Fayoumi (IDC♂×Fay♀)		Dwarf-Leghorn (IDC ♂× WLH ♀)		
	Farmer 1	Farmer 2	Farmer 3	Farmer 4	
Sutiakhali	6	6	6	6	
Boyra	6	6	6	6	
Kewatkhali	6	6	6	6	
Total		36		36	

^{*}IDC-indigenous dwarf chicken, Fay-Fayoumi, WLH-White Leghorn, ♂=male; ♀=female

2.3. Housing and management

Each mini shed was made of wood, bamboo, wire net, polythene and CI sheet or sometimes paddy straw as roof materials. Pen size was 1.5 m^2 ($1.5 \text{ m} \times 1 \text{ m}$) and provision of floor space was 0.21 and 0.25 m^2 per grower and layer bird respectively. Feed and water troughs were also made of local materials or old plastic or aluminum basins. Pullets were reared on rice husk littered floors from 12^{th} to 28^{th} week under intensive management condition. Commercial poultry mash for growers (16% CP and 2700 kcal ME/kg) and layer (18% CP and 2750 kcal ME/kg) from Quality Feed Ltd. were fed to the birds. Grower and layer ration was provided as per requirements of light birds and water provided *ad libitum* for both the genotypes. Deworming drugs and vitamins were given intermittently.

2.4. Record keeping

During rearing period, live weight at different stages, feed intake, feed conversion, growth rate and survivability of birds were recorded. The age at which two hens out of five had started laying in a farmer house, was considered as the age at sexual maturity and was estimated separately for both genotypes. The production characteristics included hen-day egg production, egg weight, weight of 1st egg, egg mass, feed intake, FCR, age and weight at sexual maturity were assessed and calculated accordingly (Amao *et al.*, 2015; Anene *et al.*, 2020). Egg production was recorded daily at evening in a pre-designed record sheet for each flock. All sorts of egg production data were recorded up to 28th week from the onset of laying. Survivability was calculated from mortality data. Mortality was recorded in different age groups (13-20, 21-28 and 13-28 weeks).

2.5. Data analysis

The collected data was compiled in excel sheet of MS office from the record sheet maintained during the experimental period. The data was then processed through sorting and removing of extreme value. Data were then analyzed using analysis of variance technique using SAS statistical package in accordance with the principle of completely randomized design (SAS Institute, 2014). Paired *t*-test was performed to know significance level between genotypes for different traits.

3. Result

3.1. Growth performance

There were highly significant differences (P<0.001) observed in the live weights between IDC \circlearrowleft × Fay \circlearrowleft and IDC \circlearrowleft × WLH \circlearrowleft pullets at all ages. The live weights of IDC \circlearrowleft × Fay \circlearrowleft pullets were 623.81 ± 6.18, 849.59 ± 7.0 and 1039.50 ± 8.64 g at 12, 16, and 20 weeks of age and the corresponding values were 697.58 ± 6.08, 919.64 ± 7.39 and 1103.92 ± 9.52 g, respectively in IDC \circlearrowleft × WLH \circlearrowleft pullets. The results revealed differences (P<0.01) also in feed intake and feed conversion ratio (FCR) across the genotypes favoring the IDC \circlearrowleft × Fay \hookrightarrow pullets at 13th-16th weeks of age. IDC \circlearrowleft × Fay \hookrightarrow and IDC \circlearrowleft × WLH \hookrightarrow pullets consumed 60.79 ± 0.46 and 62.98 ± 0.39 g feed daily and the resultant FCR was 7.55 ± 0.06 and 8.02 ± 0.08 at 13th-16th weeks of age. However, the parameters mentioned ahead were similar across the two studied genotypes during the period of 17th-20th weeks. The rate of growth however did not vary between the genotypes (Table 2).

Table 2. Growth performance of upgraded dwarf chicken under farmers' condition.

Trait	Age (weeks)	Mean ± SE		Significance level
		Dwarf-Fayoumi (IDC♂×Fay♀)	Dwarf-Leghorn (IDC ♂× W L H ♀)	
Live weight (g/bird)	12	623.81 ± 6.18 (36)	$697.58 \pm 6.08 (36)$	***
	16	$849.59 \pm 7.0 (34)$	919.64 ± 7.39 (28)	***
	20	$1039.50 \pm 8.64 (34)$	1103.92 ± 9.52 (26)	***
Growth rate	13-16	8.05 ± 0.06 (34)	7.87 ± 0.09 (28)	NS
(g/day/bird)	17-20	6.78 ± 0.09 (34)	$6.74 \pm 0.09 (26)$	NS
Feed intake	13-16	60.79 ± 0.46	62.98 ± 0.39	**
(g/day/bird)	17-20	69.15 ± 0.45	70.08 ± 0.35	NS
Feed conversion	13-16	7.55 ± 0.06	8.02 ± 0.08	**
efficiency (FCE)	17-20	10.21 ± 0.12	10.39 ± 0.06	NS

Values= Mean \pm SE, in the parenthesis the N values are presented; IDC-Indigenous Dwarf Chicken, Fay-Fayoumi, WLH-White Leghorn, SE-standard error, significant level at **P<0.01, ***P<0.001, NS-non significant.

The results also indicated that the adaptability (accessed through the survivability) varied significantly between the genotypes with the values being better among the IDC $\circlearrowleft \times$ Fay \circlearrowleft individuals. Survivability of IDC $\circlearrowleft \times$ Fay \circlearrowleft and IDC $\circlearrowleft \times$ WLH \circlearrowleft pullets were ranged between 94.44 \pm 4.72% and 72.22 \pm 3.61% during the experimental period (Figure 2).

3.2. Production and reproduction potentialities

Highly significance differences were observed in egg mass $(25.07 \pm 0.20 \text{ g/bird/day})$, hen day egg production $(37.48 \pm 0.43\% \text{ up to } 28 \text{ weeks of age})$, egg-feed price ratio (1.18 ± 0.02) favoring (P<0.001) in IDC $\circlearrowleft \times \text{Fay} \hookrightarrow \text{Fay}$. Performance efficiency index (21.22 ± 0.95) was also found significantly better (P<0.05) in IDC $\circlearrowleft \times \text{Fay} \hookrightarrow \text{Pay}$ pullets. On the other hand, lower age at sexual maturity $(147.50 \pm 0.76 \text{ days})$ and feed intake $(74.55 \pm 0.23 \text{ g})$,

and higher FCR (2.98 \pm 0.02) were observed among the IDC $\circlearrowleft \times$ Fay \circlearrowleft (P<0.001). The findings also indicated that the weight at sexual maturity (1153.33 \pm 4.33 g) and 1st egg (34.17 \pm 0.31) favored (P<0.001) the IDC $\circlearrowleft \times$ WLH \circlearrowleft pullets. The study also indicated that the average egg weight also differed significantly (P<0.01) between these two genotypes, 40.92 \pm 0.15 g and 41.78 \pm 0.22 g for IDC $\circlearrowleft \times$ Fay \circlearrowleft and IDC $\circlearrowleft \times$ WLH pullets, respectively (Table 3).

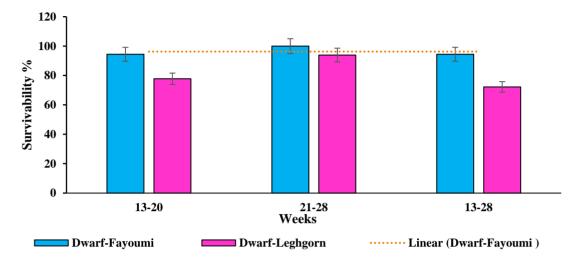


Figure 2. Comparison of survivability (%) in between two upgraded dwarf genotypes under farmer's condition.

Table 3. Production and reproduction potentialities of upgraded dwarf chicken under farmers' condition up to 28 weeks of age.

Trait	Mean ± SE		Significance level
	Dwarf-Fayoumi (IDC♂×Fay♀)	Dwarf-Leghorn (IDC♂×WLH♀)	
Age at sexual maturity (days)	147.50 ± 0.76	169.83 ± 1.35	***
Weight at sexual maturity (g)	1101.5 ± 3.96	1153.33 ± 4.33	***
Weight of 1 st egg (g)	28.17 ± 0.31	34.17 ± 0.31	***
Average egg weight [§] (g)	40.92 ± 0.15	41.78 ± 0.22	**
Egg mass [§] (g/bird/day)	25.07 ± 0.20	21.71 ± 0.11	***
Feed intake [§] (g)	74.55 ± 0.23	76.07 ± 0.19	***
Feed conversion ratio§	2.98 ± 0.02	3.51 ± 0.02	***
Hen day egg production [§] (%)	37.48 ± 0.43	31.85 ± 0.25	***
(up to 28 weeks of age)			
Hen day egg production (%)	61.26 ± 0.26	51.96 ± 0.35	***
(at 28 th week)			
Performance efficiency index§	21.22 ± 0.95	18.49 ± 0.40	*
Egg-feed price ratio [§] (EFPR)	1.18 ± 0.02	1.00 ± 0.01	***

IDC-Indigenous Dwarf Chicken, Fay-Fayoumi, WLH-White Leghorn, SE-standard error, significant level at *P<0.05, **P<0.01, ***P<0.001, **P<0.001, ***P<0.001, ***P<0.001, ***P<0.001, ***P<0.001, **P<0.001, **

The study revealed that $IDC \nearrow Fay \supsetneq$ pullets showed earlier sexual maturity and lower feed intake and thus had a better FCR during the experimental period. Moreover, among the production traits considered egg mass, hen day egg production, performance efficiency index and egg feed price ratio were significantly higher among the $IDC \nearrow Fay \supsetneq$ pullets. During the experimental period, the $IDC \nearrow Fay \supsetneq$ pullets survived better than the $IDC \nearrow V$ with $\nearrow V$ counterparts.

4. Discussion

The live weights of IDC $\circlearrowleft \times \text{Fay} \supsetneq$ pullets were 623.8, 849.5, 1039.5 g at 12, 16, and 20 weeks of age, respectively and were 697.5, 919.6, 1103.9 g, respectively in IDC $\circlearrowleft \times \text{WLH} \supsetneq$ pullets. Live weights of IDC $\circlearrowleft \times \text{WLH} \supsetneq$ pullets were higher (P < 0.001) than those of the IDC $\circlearrowleft \times \text{Fay} \supsetneq$ pullets at all ages. Findings of a study by

Singh (2002) reported that weight of Shyma chickens was 1120 g at 20 weeks of age which is in accordance with those of the present investigation. Daily feed consumption of IDC \circlearrowleft × Fay \circlearrowleft and IDC \circlearrowleft × WLH \circlearrowleft pullets were recorded as 60.7 and 62.9 g and FCR were 7.55 and 8.02 during13th to16th weeks of age. Lower feed intake and higher feed conversion efficiency was recorded among the IDC \circlearrowleft × Fay \circlearrowleft pullets than IDC \circlearrowleft × WLH \circlearrowleft pullets. Better feed conversion by relatively dwarf pullets as recorded in this experiment is in close accordance with the findings of Yeasmin and Howlider (2013); Osei-Amponsah *et al.* (2015); Ferdaus *et al.* (2016) and Yi *et al.* (2018).

The results from the study indicated that the age at sextual maturity was lower among the IDC \circlearrowleft × Fay \subsetneqq pullets (147.5 days) when compared to IDC \circlearrowleft × WLH \subsetneqq pullets (169.8 days). Similar findings were also reported by Bahry *et al.* (2023) who found lower age at sexual maturity among Lohmann LSL lite (W) and Lohmann brown lite (B) breed. The findings of Khawaja *et al.* (2013) are similar to the present findings who indicated that sexual maturity was lowest in the Rhode Island Red \circlearrowleft × Fayoumi \subsetneqq (RIFI), Fayoumi \circlearrowleft × Rhode Island Red \subsetneqq (FIRI) and White Leghorn \circlearrowleft × FIRI \subsetneqq (RLH). The delayed sexual maturity among the IDC \circlearrowleft × WLH \subsetneqq chicken is also in consonance with those of Bahry *et al.* (2023), van der Klein *et al.* (2018) and van der Klein *et al.* (2020). The results also indicated that heavier breeds of chickens have a delayed maturity adversely influencing total egg production and egg mass.

Weight at sexual maturity was found to be lower among the IDC \circlearrowleft × Fay \subsetneqq (1101.5 g) when compared to the IDC \circlearrowleft × WLH \subsetneqq pullets (1153.3 g) which is in agreement with the findings of Yeasmin (2001). Weight of the first egg laid (28.17 g) and average egg weight (40.92 g) were also lower among the IDC \circlearrowleft × Fay \subsetneqq pullets which is in close accordance with the findings of Padhi *et al.* (2016), Atsbeha and Hailu (2021) and Khawaja *et al.* (2012). However, egg mass was higher among the IDC \circlearrowleft × Fay \subsetneqq pullets (25.07 g/bird/day) when compared to the IDC \circlearrowleft × WLH \subsetneqq counterparts (21.71 g/bird/day). This finding is partially in agreement with the findings of Anene *et al.* (2020) and Araújo *et al.* (2015). Egg mass production under farmer's condition depends on the availability of the feed ingredients for scavenging as well as the amount of feed supplementation.

In the present study, lower feed intake (74.55 g) and better feed efficiency (2.98) was observed among the IDC $\circlearrowleft \times \operatorname{Fay} \supsetneq$ pullets which in close accordance with the findings of Yeasmin *et al.* (2003). Reduced feed intake and better feed conversion efficiency of the dwarf chicken was also in consonance the findings of Ferdaus *et al.* (2016), Shafiq *et al.* (2022) and Yi *et al.* (2018). Hen day egg production of 28^{th} week (61.26%) and up to 28 weeks (37.48%) of age were higher among the IDC $\circlearrowleft \times \operatorname{Fay} \supsetneq$ pullets when compared to those of IDC $\circlearrowleft \times \operatorname{WLH} \supsetneq$ pullets which too is in close accordance with those of Hasan *et al.* (2021).

Performance efficiency index (PEI) was found to be higher among the IDC $\circlearrowleft \times \text{Fay} \supsetneq$ pullets (21.22) vis-a-vis those of IDC $\circlearrowleft \times \text{WLH} \supsetneq$ pullets (18.49) in the present investigation. The findings of a study by Cheraghi *et al.* (2014) and Nath *et al.* (2022) were similar to the present study. PEI of all of the above findings were higher than present findings. Shorter rearing period (up to 28 weeks of age) was the major limiting factor and is not justified to compare with others using incomplete data set.

Higher egg-feed price ratio (EFPR) was found (P<0.001) in IDC \circlearrowleft × Fay \circlearrowleft pullets (1.17) than those of IDC \circlearrowleft × WLH \circlearrowleft pullets (0.99) in the present study. EFPR was 17.9% higher among the IDC \circlearrowleft × Fay \circlearrowleft pullets when compared to those of the IDC \circlearrowleft × WLH \circlearrowleft pullets. The findings of Ojediran *et al.* (2022) reported that EFPR varied from 1.76 to 3.37 across different genotypes of chickens. Mensah (2016) found that chickens raised in medium-scale farms exhibited higher EFPR (1.64) compared to those in small-scale farms (1.37). Their findings were higher than the current findings. The EFPR was lower in the current study due to shorter rearing period compared to above findings.

Higher survivability during $13^{th}-28^{th}$ weeks was recorded among the IDC $^{\wedge}$ × Fay $^{\wedge}$ (94.4%) pullets when compared to those of IDC $^{\wedge}$ × WLH $^{\wedge}$ pullets (72.2%). However, the lower mortality among the smaller dwarf birds, as recorded in a study by Uddin *et al.* (2012), is in close accordance with the present findings. Hailegebreal *et al.* (2022) reported that mortality among the improved breeds of chickens till 8 weeks of age was 1.61%. The mortality among the IDC $^{\wedge}$ × Fay $^{\wedge}$ chicken in the current study is very close to the findings of Hailegebreal *et al.* (2022). The increased survivability and adaptability of crossbreds compared to purebreds of chicken, as reported by Jahan *et al.* (2021), is close to the present findings. However, our datasets represented only upgraded genotypes and therefore, is not justify to compare with pure breed chicken. The study also indicated that under farm conditions of Bangladesh IDC $^{\wedge}$ × Fay $^{\wedge}$ chickens adapted better when compared to the IDC $^{\wedge}$ × WLH $^{\wedge}$. These findings too are in close accordance with those from the previous studies which indicated that Fayoumi chickens have a better adaptability vis-a-vis those of White Leghorns at the farmer's level and therefore, the upgraded genotypes with Fayoumi genotypes have better adaptability to hot and humid condition of Bangladesh (Balcha *et al.*, 2021; Negash *et al.*, 2023).

4. Conclusions

The study revealed that the parameters involved with performance and revenue were found to be higher in Dwarf-Fayoumi pullets. In addition, higher survivability was observed in Dwarf-Fayoumi pullets than those of Dwarf-Leghorn pullets. Based on performance records, it is evident that all considered traits were found to favor in Dwarf-Fayoumi pullets. Therefore, it can be concluded that the Dwarf-Fayoumi pullets are more productive, profitable and adaptable when compared to Dwarf-Leghorn pullets under small holder farmers' condition. This study provides some basic and needful information about the potentialities of upgraded dwarf genotypes which could be utilized as mini layer under semi-scavenging system of Bangladesh.

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Data Availability

All the necessary data used in this research will be made available as per the authorization of the authors.

Conflict of interest

None to declare.

Authors' contribution

Abu Jafur Md. Ferdaus: Conducted the research and prepared the draft manuscript. Begum Mansura Hassin: Assisted the research and manuscript preparation. A S M Atiquzzaman: Critically evaluate the manuscript. Mohammad Shamsul Alam Bhuiyan: Conceptualization, design of the study, methodology, writing-review and editing. Md. Shawkat Ali: data analysis and reviewed the manuscript. All authors have read and approved the final manuscript.

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