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Influence of Energy Restriction and Pre-incubation Holding Period of Eggs on Fertility and Hatchability in Aged Broiler Breeders

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ABSTRACT : The effects of controlled energy restriction and duration of pre-incubation egg holding on fertility, hatchability and hatch losses were evaluated in aged broiler breeders (64 wk). The energy (ME) required for maintenance, activity, growth and anticipated egg production was calculated and offered to a control group (283-471 kcal/kg) from 21-64 weeks of age. In three other groups, ME was quantitatively reduced either by 20% (SER; severe energy restriction) or 10% (MER; moderate energy restriction) and increased by10% (EEF; excess energy feeding) over the control group (CER; controlled energy restriction). Each diet was offered to 130 pullets in individual cages, and the quantity of ME increased with age. At the end of 64 weeks, fertile eggs were collected from each dietary group for 11 consecutive days and grouped under 4 holding periods based on the length of storage (2, 5, 8 or 11 d). The influence of energy regimes, egg holding intervals and their interaction was evaluated on fertility, hatch losses and hatchability. Broiler breeders maintained on SER regime (231-419 kcal/d) produced maximum number of eggs (993) followed by MER (819), CER (624) and EEF (438) during the 11-day period. The percent fertility and hatchability was significantly ($p \leq 0.05$) higher in SER and MER groups compared to CER and EEF. However, energy regimes did not influence the loss in egg weight during pre-incubation storage, shell weight, shell thickness or hatch losses as dead germs and dead in shell. The improvement in hatchability in SER and MER groups appeared to be closely related to higher fertility and lower embryonic mortality. Holding of eggs for 11 days showed a linear loss in egg weight with the length of storage, but did not influence the fertility and hatch losses. The percent hatchability on eggs set was maximum when storage period was restricted to 5 days. The interaction between energy regimes and egg holding periods exhibited better hatchability results with SER regime when eggs were held for 5 days. Response to MER was not different from SER. It was obvious that energy restriction during production period had a positive influence on egg number, fertility and hatchability in aged breeders. At 64 weeks of age, holding of fertile eggs for 5 days prior to incubation was adequate for optimum hatchability in breeders. (**Key Words :** Energy Restriction, Pre-Incubation Holding, Fertility, Hatchability, Aged Broiler Breeders)

INTRODUCTION

Broiler breeders are sensitive to free allowances of feed. Excess feeding of breeders negatively affected egg production, shell quality, fertility and hatchability (Mc Daniel et al., 1981). Broiler breeders with access to *ad libitum* feeding consume energy (643 kcal/d), which is almost twice the quantity of actual requirement (370 kcal/d) for maintenance and egg production (Lopez and Leeson, 1994). Therefore, feed restriction during rearing and laying periods was effective in considerably reducing erratic oviposition and improving the number of settable eggs, even during later period (62 wk of age) of production (Yu et al., 1992). Further, feed restriction improved the duration of fertility (12.7 d) compared to full feeding (10 d) (Goerzen et al., 1996). The fertile eggs collected from broiler breeders are often stored under controlled temperature and humidity for variable lengths of time depending upon the operational requirements of the hatchery. Storage of eggs decreased the hatchability and the extent of decline is related to the length of storage period (Meijerhof, 1992) and it was observed that fertile eggs stored beyond 7 days reduced hatchability by 0.5% per day (Tandron et al., 1983). However, irrespective of storage conditions, both hatchability and chick weight varied with hen's age. The decline in hatchability in aged breeders was related to poor quality of cuticle, thin egg shells and shell membranes causing late embryonic death of germs (Ruiz and Lunam, 2002). The magnitude of decline in fertility and hatchability is influenced by the breeder nutrition, pre-incubation holding period, quality of eggs and more importantly the age of breeders. However, in aged

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breeders the hatch losses can be minimized by regulating body weight during production period and egg holding period prior to incubation. In the current study, an attempt was made to improve egg production in breeders up to 64 weeks of age through measured energy feeding, and optimize egg holding period to minimize hatch losses.

MATERIALS AND METHODS

Livestock and test diets

A total of 520 broiler pullets (20 wks) were utilized in the current feeding trial. They were housed in individual California cages (37.5×30×30 cm) in open sided house. The broiler pullets belonged to a synthetic dam line, which was evolved from the combination of White Plymouth Rock and Cornish lines. The breeders were equally allotted to 4 dietary regimes that varied in the quantity of energy (ME) fed. In the control group (CER; controlled energy restriction), measured amount of energy was offered by calculating the ME required for maintenance, activity, weight gain and anticipated egg production following the model suggested by Scott et al. (1982). The details of ME calculation for different activities are clearly illustrated in our earlier publication (Sunder et al., 2008). In two other dietary groups, the quantity of energy was reduced either severely (SER; severe energy restriction) by 20% or moderately (MER; moderate energy restriction) by 10% over the control group (CER). Further, in the fourth group, 10% excess ME (EEF; excess energy feeding) was offered over CER. At the end of 20th week, the quantity of ME allocated to SER, MER, CER and EEF groups was 231, 257, 283 and 309 kcal/bird/day, respectively. The amount of ME was increased each week, considering the criteria referred earlier, and in the 64th week the breeders in SER, MER, CER and EEF groups received 419, 445, 471 and 497 kcal/d, respectively. The quantitative variation in ME restriction was accomplished by offering measured amounts of breeder ration that contained 168 g protein and 2,600 kcal ME/kg (Table 1). The decrease of ME in SER and MER groups led to proportionate reduction in protein and amino acid, but the minimum protein required was provided to all the groups. Necessary care was taken to fortify minerals and vitamins by stepping up their supplementation in SER and MER diets and stepping down in EEF and CER diets to ensure uniform intake by all groups. The test diets were formulated using maize, soybean meal, deoiled sunflower cake meal and deoiled rice bran considering the ME values determined by Rama Rao et al. (2006) and analyzed for their proximate composition (AOAC, 1995). All the pullets were provided a single lighting regime of 14 L:10 D from 20 to 64 weeks of age. Water was provided *ad libitum*.

Table 1. Ingredient and nutrient composition (g/kg) of broiler breeder

Ingredient	Composition
Yellow maize	600.0
Soybean meal	180.0
Sunflower cake meal	100.0
De-oiled rice bran	5.4
Salt	3.0
Oyster shell grit	90.0
Dicalcium phosphate	15.0
DL-methionine	0.5
Choline chloride (50%)	2.6
Constants	3.50[1]
Nutrients	
ME (kcal/kg)[2]	2,590
Crude protein[3]	168.0
Lysine[2]	7.7
Total sulphur amino acids[2]	5.8
Calcium[3]	32.6
Avail. phosphoorus[3]	6.0
Choline[2]	1.3

[1] Constants per kg breeder diet contained: Thiamine 1.2 mg, riboflavin 13.0 mg, niacin 75 mg, pyridoxine 2.4 mg, Ca pantothenate 12.0 mg, cyanocobalamine 0.012 mg, retinol acetate 10.9 mg, cholecalciferol 0.096 mg, tocopherol acetate 13.7 mg, vitamin K 2.0 mg. Trace minerals-Fe 45 mg, Mn 60 mg, Zn 35 mg, Cu 3 mg, I 0.04 mg, Se 0.05 mg.
[2] Calculated values.
[3] Estimated values.

Egg parameters

Breeders maintained under controlled ME feeding were inseminated after 64 weeks of age with pooled semen collected from the males of the same line. Hatching eggs were collected for 11 consecutive days from breeders that were maintained under four energy restriction regimes. Their identity was established on all days of collection. Fertile eggs were weighed (g) on the day of collection and prior to incubation for calculating the percent weight loss during storage. A representative sample of 20 eggs from each dietary group was broke open in the 64th week to measure the shell weight and shell thickness using micrometer gauge (Mitutoyo, Code7027, Japan).

Pre-incubation holding of fertile eggs

Fertile eggs were collected from the 3rd day of insemination for 11 days and stored in walk-in cooler with temperature maintained at 15°C and humidity >75%. The second insemination was carried out on the 6th day. The eggs collected during the initial 3 days were stored for the longest duration of 9-11days and were considered as a single group. Similarly, the eggs pooled over for the days between 4-6, 7-9 and 10-11 remained in cold store for 6-8, 3-5 and 1-2 days, respectively. The fertile eggs collected from each dietary group for all the holding intervals were identified and accordingly set in the incubator (M/S Dayal Incubators, India) and transferred to the hatcher on the 19th day. Eggs were candled on the 3rd and 18th day to screen out infertiles and dead germs, respectively. The dead in shell embryos were separated from the healthy chicks on the 22nd day. The number of infertiles, dead germs and dead in shell were calculated to arrive at the percent hatchability on total eggs set. In addition, the percent hatchability on fertile eggs transferred was also calculated.

Statistical analysis

The data collected on different parameters were analysed for the effects of restricted energy regimes and the pre-incubation holding intervals in a two-way analysis of variance. The interaction was calculated following the completely randomized design (Snedecor and Cochran, 1989) and the mean values ($p \le 0.05$) were compared using Duncan multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Energy restriction regimes on hatchability

The number of fertile eggs collected during the entire 11 day period varied from 438 to 993 eggs in four dietary groups. The lowest egg number (438) was recoded in EEF group, which was maintained on 10% excess energy over CER (Table 2). In contrast, broiler breeders fed 20% less energy than CER produced maximum number of eggs (993), followed by MER (819) and CER (624) groups, indicating the influence of energy restriction even during later period of production at 64 weeks of age. It was possible that better persistency of egg production in SER group was due to elevated concentration of LH and FSH in plasma (Renema et al., 1999), while poor egg production in EEF group was perhaps related to excess body weight of breeders (4,487 g-at 64 wks) and early ovarian regression compared to energy restricted groups (Yu et al., 1992).

The mean egg weight was significantly less in breeders fed excess energy (EEF) compared to the energy restricted groups. It was well established that egg weight increased with age, which was primarily associated with higher yolk deposition (Roque and Soares, 1994). It was possible that CER supported greater yolk synthesis for better egg weight than SER or EEF at 64 weeks of age. The percent loss in egg weight (0.52-0.56%) during the pre-incubation storage, percent shell weight and absolute shell thickness were not influenced by the energy regimes tested in this study. However, the percent fertility was significantly high in breeders maintained on SER and MER feeding schedules compared to CER and EEF groups, implying that fertility was sensitive to energy surpluses. Breeders offered *ad libitum* feed suffered from reproductive insufficiency (Robinson et al., 1996) due to reduction in duration of fertility (21.2%) compared to those on restricted feeding (Goerzen et al., 1996). Although in our study *ad libitum* feeding was not tested, fertility in breeders of EEF group was significantly lower than the energy controlled or restricted groups, indicating the implications of even marginal energy shifts on fertility. However, energy regimes tested in this trial had no significant effect on hatching losses recorded as dead germs and dead in shell, but the

Table 2. Effect of energy restriction regimes on body weight, egg and shell parameters, fertility, hatchability, chick weight and egg: chick ratio in aged broiler breeders (64 wk)

Parameters	Energy regimes				SEM	p value
	SER[1]	MER[2]	CER[3]	EEF[4]		
Eggs set (no)	993	819	624	438	-	-
Body weight (g)	3,609[d]	3,961[c]	4,276[b]	4,487[a]	28.6	0.0001
Avg. egg wt (g)	65.48[b]	66.13[ab]	66.23[a]	64.81[c]	0.112	0.0001
Loss in egg wt (%)	0.538	0.536	0.559	0.523	0.006	0.437
Shell wt (%)	8.62	8.43	8.69	8.68	0.05	0.319
Shell thickness (mm)	0.364	0.370	0.375	0.366	0.002	0.45
Fertility (%)	90.75[a]	90.70[a]	85.39[b]	84.83[b]	0.830	0.007
Dead germ (%)	10.13	11.39	11.80	13.60	0.704	0.380
Dead in shell (%)	2.89	3.05	4.55	2.51	0.436	0.373
Hatchability on TES* (%)	78.64[a]	76.53[a]	70.51[b]	70.37[b]	1.05	0.006
Hatchability on FET** (%)	96.60	96.70	94.64	97.62	0.505	0.202
Day old chick wt (g)	45.24[a]	45.59[a]	45.58[a]	44.65[b]	0.086	0.003
Egg: chick conversion (%)	69.09	68.94	68.82	68.89	0.436	0.312

[a, b, c] Means within a row carrying different superscripts are statistically significant ($p \le 0.05$).
[1] SER- severe energy restriction (-20%). [2] MER-moderate energy restriction; (-10%).
[3] CER-controlled energy restriction. [4] EEF- excess energy feeding (+ 10%).
* TES = Hatchability on total eggs set. ** FET = Hatchability on fertile eggs transferred.

Table 3. Influence of pre-incubation holding of eggs on weight loss, fertility, hatchability, chick weight and egg to chick ratio in aged broiler breeders (64 wk)

Parameters	Pre-incubation egg holding period (d)				SEM	p value
	2	5	8	11		
Egg set (no)	486	767	847	774	-	-
Avg egg wt (g)	66.01	65.65	65.66	65.70	0.112	0.299
Loss in egg wt (%)	0.361[d]	0.418[c]	0.571[b]	0.771[a]	0.006	0.0001
Fertility (%)	89.86	89.54	88.54	84.37	0.830	0.06
Dead germ (%)	13.77	10.51	11.04	12.29	0.703	0.433
Dead in shell (%)	3.07	2.77	3.31	3.79	0.436	0.859
Hatchability on TES* (%)	77.16[a]	76.55[a]	74.58[ab]	68.82[b]	1.05	0.017
Hatchability on FET** (%)	96.89	96.88	96.24	95.71	0.505	0.819
Day old chick wt (g)	45.59	45.14	45.13	45.61	0.086	0.170
Egg: chick conversion (%)	69.06	68.76	68.73	69.42	0.437	0.526

[a, b, c, d] Means within a row carrying different superscripts are statistically significant ($p \leq 0.05$).
* TES = Hatchability on total eggs set. ** FET = Hatchability on fertile eggs transferred.

percent hatchability on total eggs set was significantly better in SER and MER fed groups than CER and EEF. Improved hatchability in the former groups appeared to be closely related to higher percent fertility and minimum embryonic losses. Harms et al. (1999) observed that breeders maintained on 475 kcal/d recorded low hatchability compared to 440 or 405 kcal/d, suggesting that optimization of energy allowances during production period was essential for increasing the chick production per breeder. In our study, breeders offered 419 or 445 kcal ME/day in the 64 week maintained better egg production, percent fertility and hatchability than CER and EEF groups fed 471 and 497 kcal ME/d, respectively corroborating the results of Harms et al. (1999). It was further seen that the weight of chick after hatch was significantly higher in groups maintained on SER, MER and CER energy regimes over EEF, implying that the egg weight at 64 weeks influenced the chick weight. However, egg to chick ratio remained the same in all groups and was not altered by energy regimes tested.

Pre-incubation holding of eggs on hatchability

The number of eggs collected for 2, 5, 8 and 11 days and retained in cold store prior to incubation were 486, 767, 847 and 774, respectively (Table 3). The average initial egg weight for different storage intervals did not vary significantly, but a linear loss in egg weight was noticed as the length of storage increased. Eggs stored for maximum period up to 11 days had no effect on percent fertility or embryonic losses as dead germs and dead in shell. However, the hatchability on total eggs set was significantly high when storage period was restricted to 5 days compared to 11 days. Although holding of eggs up to 8 days was not statistically different from 2 and 5 day-intervals, the hatchability declined by 2.0- 2.5% over the former. Brake (1995) observed that holding fertile eggs for extended periods influenced the loss of moisture and raised albumen pH to 9.0 within 4 days of storage, which was primarily due to the release of CO_2 leading to liquefaction of albumen. The changes were accelerated when eggs were held beyond 7 days (Lapap et al., 1999), causing the blastoderm to move closer to egg shell resulting in early embryonic death from dehydration (Brake et al., 1993). Similar effects were possible in our study due to significant loss in egg weight and consequently the hatchability on total eggs set, when eggs were held beyond 5 days. Although Meijerhof (1992) indicated that hatchability declined only after 7 days of storage, our findings limited the pre-incubation holding period to 5 days, expectedly due to the age of breeders (64 wk).

Energy regimes x storage intervals on hatchability

The average egg weight prior to holding them under cold storage remained unchanged within each dietary group and among the three energy restricted groups (SER, MER or CER), while excess energy feeding reduced egg weight. However, the loss in egg weight during storage was significantly influenced by the interaction between energy regimes and egg holding intervals (Table 4). Eggs from the breeders fed different energy levels exhibited similar pattern in loss of egg weight due to extended storage period, implying that weight loss was related more to the holding period than energy restriction. The percent hatchability on eggs set was significantly high in groups maintained on SER and MER regimes when held under storage for 5 days compared to the eggs from CER and EEF groups stored for 11 days. The combined effect of excess energy feeding and prolonged storage period significantly reduced the hatchability on total eggs set compared to energy restriction and shorter egg holding periods. Other combinations of energy regimes and holding periods maintained hatchability at intermediary levels. It was observed that eggs produced

Table 4. Interaction between energy regimes and pre-incubation egg holding intervals, and its influence on egg weight, fertility, hatchability, weight of day old chick and egg to chick conversion in broiler breeders after 64 wk of age

Energy regimes	Egg holding duration (d)	Loss in egg wt (%)	Fertility (%)	Hatchability TES* (%)	Day old chick wt (g)	Egg:Chick wt (%)
SER	2	0.37[de]	91.53	77.18[ab]	45.14[abcd]	68.55
	5	0.40[cd]	92.70	82.97[a]	45.18[abcd]	69.12
	8	0.57[b]	91.32	79.00[ab]	45.06[abcd]	68.73
	11	0.78[a]	87.73	74.94[ab]	45.50[abc]	69.70
MER	2	0.38[de]	91.26	75.81[ab]	45.69[abc]	68.89
	5	0.45[c]	94.77	81.17[a]	45.55[abc]	68.88
	8	0.57[b]	89.40	76.55[ab]	45.55[abc]	69.08
	11	0.74[a]	87.57	72.36[abc]	45.76[ab]	69.28
CER	2	0.38[de]	86.20	77.11[ab]	45.62[abc]	68.12
	5	0.47[c]	84.96	70.18[abc]	45.15[abcd]	68.33
	8	0.60[b]	88.55	71.04[abc]	45.38[abc]	68.72
	11	0.78[a]	82.11	65.91[bc]	45.85[a]	69.30
EEF	2	0.33[e]	90.45	78.55[ab]	44.64[bcd]	68.20
	5	0.39[de]	85.76	71.89[abc]	44.55[cd]	68.27
	8	0.56[b]	84.91	71.72[abc]	44.10[d]	68.12
	11	0.80[a]	80.08	62.05[c]	44.99[bcd]	70.17
SEM		0.006	0.830	1.05	0.086	0.436
p value		0.0001	0.08	0.01	0.03	0.892

[a, b, c, d, e] Means within a column carrying different superscripts are statistically significant ($p \leq 0.05$).
[1] SER = Severe energy restriction (-20%). [2] MER = Moderate energy restriction; (-10%).
[3] CER = Controlled energy restriction. [4] EEF = excess energy feeding (+10%).
* TES = Hatchability on total eggs set.

by aged breeders had higher egg shell conductance and poor albumen quality, which facilitated quick escape of CO_2 affecting the buffering capacity of the egg (Reis et al., 1997). This was further aggravated with extended storage periods, resulting in hatching losses (Lapap et al., 1999). The hatchability was also negatively affected by the poor quality of cuticle and shell membranes in older breeders that provided free access to bacterial penetration (North and Bell, 1990). Further, it was noticed that the content of myristic acid and linoleic acid in yolk was positively related to hatchability and these fatty acidsdecreased with age inducing late embryonic mortality (Dikmen and Sahan, 2009). It was possible in our study that egg storage for shorter duration (5 d) coupled with controlled energy feeding (SER and MER) complimented each other and restricted the decline in albumen quality for improved hatchability in aged birds. However, the interaction between energy regimes and egg holding intervals had no impact on percent fertility, dead germs, dead in shell, hatchability over eggs transferred and egg to chick ratio.

CONCLUSION

The present study revealed that energy restriction (231-419 kcal/d) in broiler breeders from 21-64 weeks had significant influence on egg production, fertility and hatchability after 64 weeks of age. Pre-incubation holding (15°C) of eggs for 5 days produced optimum hatchability, while storage for longer duration (11 d) exhibited negative impact on chick production. The combination of SER (231-419 kcal/d) or MER (257-445 kcal/d) regimes with egg holding period of 5 days was effective in producing maximum number of chicks per breeder after 64 weeks of age.

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