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Effect of Incorporating Early Feather Restriction Gene (K^S) From Native Chicken to Purebred Chicken

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ABSTRACT: Fast growing chicken suffers more in a hot humid condition than a slow growing one. The slow feathering affects the insulation mechanism of the chicken, thus enhance birds ability to dissipate heat during heat stress to a greater extent. As a consequence, slow feathered birds are more adaptive to hot humid environmental conditions. This study was undertaken to investigate the effect of incorporating early feather restriction gene (K^S) in exotic population. A total of 34 slow feathered (SF) native chicks with K^S gene of 28-day-old were collected from different villages and markets and were reared up to sexual maturity. After attaining sexual maturity, 2 males and 8 females of SF native chicken were randomly chosen to make a di-allelic cross with rapid feathered (RF) Rhode Island Red (RIR) and Fayoumi (FY). The crossbred progeny of each cross were reared up to 105 days to assess growth, survivability, heat tolerance and carcass yield under hot humid Bangladeshi condition. The collected data were arranged in a 3(genotype)×2(feather pattern)×9(age) factorial experiment in a Completely Randomized Design (CRD) and analyzed by SAS. Among genotypes, the highest survivability was obtained in *Desi*×Fayoumi (96.7%) and in SF chicks (96.1%) and the lowest in *Desi*×RIR (91.5%) and in RF chicks (90.5%). The SF chicks exhibited an average 8% heavier live weight than that of their RF counterparts. The cloacal temperature varied among genotypes and between feathering patterns (FP); being highest in *Desi*×RIR (43^0C) and in RF chicks (41.6^0C) and lowest in RIR×*Desi* (40^0C) and in SF chicks (41.4^0C). In case of *Desi*×RIR, RIR×*Desi* and *Desi*×Fayoumi, cloacal temperature increased linearly by 0.047, 0.044 and 0.032^0C for each gram of live weight increase. Further, the cloacal temperature increased linearly by 0.036 and 0.044^0C for the increase of each gram of live weight gain in case of RF and SF chicks, respectively. There was no significant ($P>0.05$) difference of dressing percentage for the effect of either genotype or FP (55.4% for SF vs. 46.6% for RF birds). Genotype and FP had significant effects ($P<0.05$; $P<0.01$) on total meat yield; being highest in *Desi*×RIR (24.6%) and in SF birds (26.2%) and lowest in *Desi*×Fayoumi (21.4%) and in RF birds (20.6%). Breast meat yield of SF birds (9.2%) was significantly ($P<0.01$) higher than that of RF birds (7.1%). The highest thigh meat yield was obtained in *Desi*×RIR (7.6%) and in SF birds (8.3%) and the lowest in *Desi*×Fayoumi (6.7%) and in RF counterparts (6.0%). The highest drumstick meat yield was obtained in RIR×*Desi* (5.6%) and in SF birds (5.9%) and the lowest in *Desi*×Fayoumi (5.0%) and in RF birds (4.8%). Genotype and FP had significant interaction effect on total meat yield as well as fractional meat yields. The study revealed that incorporation of 'K^s' gene in the exotic one by crossing with native chicken and rearing under hot humid environment may be a means of improving growth, survivability, heat tolerance and carcass yield.

KEYWORDS: Slow feathered gene, adaptability, body weight, meat yield.

INTRODUCTION

Bangladesh is traditionally a hot humid country under Asiatic zone having high temperature and high humidity, especially in the summer. However, chicken is very much sensitive to climate and environment for their adaptation as well as production performance. Howlider and Rose (1987) have shown a remarkable diminish in growth due to elevation of environmental temperature in broiler chicken. The growth is hampered due to inability of the birds to dissipate heat when reared in a hot humid environment (Daeton and Recee, 1970). There are substantial evidences that environmental temperatures ranging from 32 to 42^0C as often encountered in summer month, cause a number of altered physiological and economic conditions in poultry. Though modern breeds, variety and strains of poultry reared either in the controlled or free range environment cannot withstand the stress of higher temperature and humidity. The modern breeds are well covered by feathers and they cannot dissipate metabolic heat produced in the body properly to its surroundings. Panting is an important means of combating heat loss. But, it is very insufficient as a means of losing heat and energy cost of panting is also higher. Therefore, poultry suffers seriously in the summer.

A fast growing chicken like broiler suffers more in a hot humid condition than a slow growing one. The problem of heat stress becomes severe when a higher temperature is accompanied by a higher relative humidity (Charles, 1986). The reduction of feed intake, growth rate and egg production are the usual consequences (Dale and Fuller, 1980; Cowan and Michie, 1978; Charles, 1986; Howlider and Rose, 1987). The slow feathering affects the insulation mechanism of the chicken, thus enhance birds ability to dissipate heat during heat stress to a greater extent. As a consequence, slow feathered birds are more adaptive to hot humid environmental conditions. Besides, the gene for naked neck (Na), a sex-linked dominant gene (K) also restricts feather growth in the early life of the chicken. The birds with "K^S" gene have plenty of scope to dissipate metabolic heat through their body surface. The K^S gene restricts feather growth in breast, back and abdomen during the early life of the chicks (Siegel *et al.,* 1957). Since birds are in higher metabolic profile in early life, they need to get rid of extra heat in hot humid climate. The K^S gene provides such facilities resulting heat tolerance to them. Investigation revealed higher meat content in the carcass of birds having K^S gene. The birds with K^S gene inheritance are also less vulnerable to feather pecking and cannibalism. They have lower dietary protein requirement during early life and also have lower mortality when reared in hot humid environments. These are the indications of higher adaptiveness of the birds with K^S gene inheritance in hot humid conditions.

The recent trend of poultry breeding is to incorporate tropical relevant genes such as naked neck (Na), slow feathering (K^S) and dwarfism (dw) to high yielding and dual purpose types to increase adaptability under tropical conditions. It was reported that "Na" and "K" are distinct genes which increase the heat tolerance in poultry. If these genes from local population are introduced to some standard breeds by hybridization technique that might give a satisfactory adaptability under the harsh and tropical environment of Bangladesh. The incidence of K^S gene is known to exist in indigenous chicken population of Bangladesh (Crawford, 1990). The effect of K^S gene on early growth and heat tolerance of indigenous stock is yet to be investigated. Therefore, this study was undertaken to investigate the effect of incorporating early feather restriction gene (K^S) from local population to exotic population by assessing growth, survivability, heat tolerance and meat yield under hot humid Bangladeshi condition.

MATERIALS AND METHODS

The experiment was conducted at the poultry farm of Bangladesh Agricultural University (BAU), Mymensingh from a total period of 15 months. For this experiment, a total of 34 slow feathered native chicks with K^S gene of 28-day-old were collected from different villages and markets and were reared up to sexual maturity (190 days). After attaining sexual maturity, 2 males and 8 females of slow feathered native chicken were randomly chosen to make a di-allelic cross with Rhode Island Red (RIR) and Fayoumi with an intention to produce 4 respective crosses. The layout of di-allelic crossing is as follows:

Pen No	Male line	Female line	Genotype
1	Slow feathered native (01)	Normal feathered RIR (04)	SFN-RIR
2	Normal feathered RIR (01)	Slow feathered native (04)	RIR-SFN
3	Slow feathered native (01)	Normal feathered Fayoumi (04)	SFN-FY
4	Normal feathered Fayoumi (01)	Slow feathered native (04)	FY-SFN

Just after collection, the slow feathered native chicks were kept in an isolation room for quarantine followed by rearing in cages ($450cm^2$/bird) and fed *adlib* on a grower dry mash ration containing 2912 Kcal/Kg ME and 16.5% CP with sufficient fresh drinking water. The parents were transferred from cage to the floor pens at 81 days of age allowing density of $900cm^2$/bird and fed on a laying dry mash containing 2819 Kcal/Kg ME and 17.3% CP. All the experimental birds were vaccinated against Fowl Pox, New castle Disease and Fowl Cholera at 45, 60 and 75 days of age.

The eggs produced from the four pens were collected separately twice in a day from each of the pen. After proper cleaning, eggs were stored at 13^0C (55^0F) before setting to hatch. The average egg productions were 52%, 40%, 58% and 35%, respectively in Pen-1, Pen-2, Pen-3 and Pen-4. For hatching, the required number of eggs laid in 4 pens were set in a forced draft incubator separately with adequate temperature and humidity and obtained hatchability of 60.83%, 55.67%, 75% and 0%, respectively.

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Finally, a total of 45 crossbred progeny from Pen-1, 35 crossbred progeny from Pen-2 and 77 crossbred progeny from Pen-3 were produced. All chicks were classified by the degree of feather development at hatching following the description of Warren (1925). The chicks in which the primary and secondary feathers at day-old projected well beyond down and wing coverts were called rapid feathered (k`), while, chicks having moderately developed primaries and secondaries, but did not projected beyond down and wing coverts were called slow feathered (K^S). The classified chicks were tagged with wing band and kept in brooding (allowing space of 500cm^2/bird) up to 4 weeks of age with adequate brooding temperature (started with 35^{0}C and decreased gradually up to 27^{0}C) and lighting (night lighting was reduced @ 30 minutes per week until the night lighting was over, while afterwards, no night light provided up to 105 days of age).

The crossbred chicks were fed *adlib* on a dry mash diet containing 2857 Kcal/Kg ME and 18.24% CP up to 28 days of age. Afterwards, they were provided on a dry mash grower ration containing 2912 Kcal/Kg ME and 16.5% CP up to 105 days of age with same management as followed in their parents. In rearing period, no artificial temperature was given. However, ambient temperature (^{0}C) and relative humidity (%RH) were recorded 4 times in a day. The cloacal temperature of each bird was also recorded in the vent using clinical thermometer at 12, 21, 35, 49, 63, 91 and 105 days of age. During rearing period, the average room temperature and relative humidity were recorded as 31.7^{0}C and 85.3%. The body weights of the experimental chicks were recorded at day-old, 12, 21, 35, 49, 63, 77, 91 and 105 days of ages. The survivability of the chicks was also recorded throughout the experimental period. To observe the meat yield on the carcass of the rapid and slow feathered chicks, 12 chicks of different groups and sexes were slaughtered after recording live weight for dissection purposes according to the procedure of Jones (1984).

The collected data were arranged in a 3 (genotype) × 2 (feather pattern) × 9 (age) factorial experiment in a Completely Randomized Design (CRD) according to Steel and Torrie (1960). Analysis of variance was performed to compare the recorded variables among genotypes, feather pattern, age and their interactions. Data were analyzed following Generalized Linear Models (GLM) procedure using SAS (1990) statistical package. The mean comparisons among variables were separated by Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

The survivability of chicks up to 105 days age among three crossbred genotypes and two feathering patterns is depicted in Table 1. In rapid feather chicks, the highest survivability (93.5%) was obtained in *Desi*×Fayoumi and the lowest (88.0%) in *Desi*×RIR with an average survivability of 90.5%, irrespective of genotypes. On the other hand, in slow feather chicks, the highest survivability (100%) was obtained in *Desi*×Fayoumi and the lowest (93.3%) in RIR×*Desi* with an average survivability of 96.1%, irrespective of genotypes. However, irrespective of feathering pattern, the highest survivability (96.74%) was obtained in *Desi*×Fayoumi and the lowest (91.50%) in *Desi*×RIR chicks. Our investigation reveals that the slow feathered chicks had 6.2% higher survivability than those of their rapid feathered one's. This finding is in agreement with Sergeev and Repina (1990) who observed higher chick survival rate in case of auto-sexing late feathering broilers than that of non-auto-sexing early feathering broilers. In contrast, Godfrey and Farnsworth (1952) and Yoshida and Saito (1983) found no detectable effect of the rapid feathering gene (k) on the survival rate of the White Leghorn birds. The variation could be due to different genotype, environment, feeding and management system in different experiments.

Live weights of rapid and slow feathered crossbred progeny up to 105 days age are illustrated in Table 2 which demonstrated that there were highly significant ($P<0.01$) differences in body weight among genotypes and between feathering patterns and also influenced by the interaction ($P<0.05$) of genotype and feathering pattern. The slow feathered chicks exhibited an average 8% heavier live weight than that of their rapid feathered counterparts. The SFN-RIR and SFN-FY slow feathered chicks had higher growth rate than their rapid feather counterparts from day-old to 105 days of age in our study were in general agreement with many other researchers (Siegel *et al.,* 1957; Sheridan and McDonald, 1963; Verma and Singh, 1983; Verma and Prasad, 1981). On the other hand, Table 2 also entailed RIR-SFN rapid feathered crossbred chicks to be higher growth rate than that of their counterpart slow feathered chicks. The results are in general agreement with the findings of Dunnington and Siegel (1986) and Merat (1959). Our result however, contradict the result of Yoshida and Saito (1983) who did not find any detectable effect of the rapid feathered gene (k gene) on the body weight of the White Leghorn chicks. But, results reviewed by Merat (1970) concerning the growth of the rapid feathering chicks showed either no difference or a slight superiority for rapid feathered chicks. Godfrey and Farnsworth (1952), Sheridan and McDonald (1963) and Merat (1970) did not find any relation between rate of feathering and live weight gain of chicks. Interaction among different rearing environment (Siegel *et al.,* 1957), variable diets differing in nutrient contents (Dunnington and and Siegel, 1986) and the various genetic background (Hurry and Nordskog, 1953) of the stocks used in different investigations may be the possible reasons for variable response of incorporating 'K' gene in chicken population. In this study, the incorporation of 'K' gene seemed to promote growth rate of SFN-RIR and SFN-FY, but depleted growth in RIR-SFN genotype than in full feathered counterparts.

The cloacal temperatures of rapid and slow feathered crossbred progeny from 12 up to 105 days of ages are shown in Table 3. The results as depicted in Table 3 revealed that cloacal temperature varied among genotypes and between feathering patterns. The cloacal temperature was found to be highest in *Desi*×RIR (43^{0}C), intermediate in *Desi*×Fayoumi (42^{0}C) and lowest in RIR×*Desi* (40^{0}C). However, a highly significant (P<0.01) difference of cloacal temperature was observed between rapid and slow feathered chicks. The rapid feather chicks had a higher (41.6^{0}C) cloacal temperature than those of their counterpart slow feathered chicks (41.4^{0}C). Higher cloacal temperature in heavier birds as observed in our finding is in agreement with Dunnington and Siegel (1986) who observed that birds with smaller body size were enabled to maintain cooler body temperature in warm environment than the larger birds. A higher cloacal temperature in rapid feathered chicks than in slow feathered chicks obtained in our observation agrees with the report of Romijin (1950), who reported the marked influence of the degree of feathering insulation on heat loss from body surface of the birds.

Table 4 represents the relationship of live weight with cloacal temperature among genotypes and feathering patterns. For all genotypes and both feathering patterns, significantly (P<0.01) higher positive correlations existed between live weight and cloacal temperature. In case of *Desi*×RIR, RIR×*Desi* and *Desi*×Fayoumi, cloacal temperature increased linearly by 0.047, 0.044 and 0.032^{0}C for each gram of live weight increase. Further, the cloacal temperature increased linearly by 0.036 and 0.044^{0}C for the increase of each gram of live weight gain in case of rapid and slow feathered chicks, respectively. The results however suggested that irrespective of genotype and feathering pattern, body weight had positive correlations with cloacal temperatures which increased linearly with increasing live weight. Such a relationship of cloacal temperature with body weight is agreed partially by Bohren *et al.* (1982), who reported higher heat stress-mortality in the fast growing chickens.

The carcass characteristics among crossbred progeny and feather patterns are depicted in Table 5 which entails that the highest dressing percentage was obtained in *Desi*×RIR (53.2%), intermediate in RIR×*Desi* (50.9%), and the lowest in *Desi*×Fayoumi (48.8%) irrespective of feathering pattern, although the differences were not significant (P>0.05). On the other hand, the highest dressing percentage was obtained in slow feathered birds (55.4%) and the lowest in rapid feathered birds (46.6%) irrespective of genotypes, although the difference was not significant (P>0.05). Table 5 also shows that genotype and feathering pattern had no interaction effect for dressing percentage. The results in our study imply that higher carcass yield for slow feathered chicks than that of rapid feathered chicks observed in this study could be due to more adaptability of slow feathered chicks than those of their counterpart rapid feathered chicks. This finding confirms the findings of previous studies of Merat (1986), Horst and Rouen (1986), Barua and Howlider (1991), Hossain *et al.* (1991) and Alam *et al.* (1995).

The total and fractional meat yields of different crossbred progeny and feathering pattern were estimated as shown in Table 5. Genotype and feathering pattern had significant effects (P<0.05; P<0.01) on total meat yield, being highest in *Desi*×RIR (24.6%) and lowest in *Desi*×Fayoumi (21.4%), irrespective of feathering pattern and 26.2 and 20.6%, respectively for slow and rapid feathered birds, irrespective of genotypes. The breast meat yield did not differ significantly (P>0.05) for the effect of genotype, while it differed significantly (P<0.01) between feathering patterns. Irrespective of genotype, breast meat yield of slow feathered birds (9.2%) was significantly (P<0.01) higher than that of rapid feathered birds (7.1%). In case of thigh meat yield, both genotype and feathering pattern had highly significant (P<0.01) influence on it. Irrespective of feathering pattern, the highest thigh meat yield was obtained in *Desi*×RIR (7.6%), intermediate in RIR×*Desi* (7.2%) and the lowest in *Desi*×Fayoumi (6.7%). On the other hand, the highest thigh meat yield was obtained in slow feathered birds (8.3%) and the lowest in rapid feather counterparts (6.0%). The drumstick meat yield influenced for the direct effect of genotype (P<0.05) and feathering pattern (P<0.01). The highest drumstick meat yield was obtained in RIR×*Desi* (5.6%), intermediate in *Desi*×RIR (5.5%) and the lowest in *Desi*×Fayoumi (5.0%) regardless of feathering pattern. On the other hand, the highest drumstick meat yield was obtained in slow feathered birds (5.9%) and the lowest in rapid feathered birds (4.8%) regardless of genotype.

The highest total and fractional meat yields in dressed carcass in favor of slow feathered birds as obtained in our study corresponds with earlier findings of Merat (1986), Horst and Rouen (1986), Barua and Howlider (1991), Hossain *et al.* (1991) and Alam *et al.*

(1995). Though, the rapid and slow feathered birds had similar (P>0.05) dressing yield, however, the later had higher total meat yield than that of earlier, which imply that the slow feathered birds had higher meat : bone in their carcasses than those of their rapid feathered counterparts. Table 5 also shows that genotype and feathering pattern had significant interaction effect on total meat yield as well as fractional meat yields, which indicates that there is opportunity for selection and breeding of best combination of genotype and feathering pattern for higher meat yields.

CONCLUSION

This study revealed that irrespective of genotypes, the slow feathered chicks exhibited a definite superiority in live weight, heat tolerance, survivability and carcass yield than those of the rapid feathered chicks. The incorporation of 'K' gene from native chicken may be a means of improving growth, survivability, heat tolerance and carcass yield to exotic to rear under hot humid environment of Bangladesh. The result however, implies that genotype should be an important consideration to exploit the advantage of 'K' gene.

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Table 1: Survivability (%) of chicks up to 105 days age among genotypes and feathering pattern

Genotype	Feathering pattern		Mean
	Rapid feathered	Slow feathered	
Desi×RIR	88.0	95.0	91.5
RIR×*Desi*	90.0	93.3	91.7
Desi×Fayoumi	93.5	100.0	96.7
Mean	90.5	96.1	93.3

Table 2: Live weight of rapid and slow feathered crossbred progeny

Genotype (G)	Feathering pattern (FP[$])	Age (A) in days									Mean	LSD (SED) and significance level[+]					
		1	12	21	35	49	63	77	91	105		G	FP	A	G×FP	G×A	FP×A
Desi×RIR	RF	36.6	53.4	89.1	115.0	165.6	277.5	330.6	444.3	598.6	228.96	8.89**	7.26**	15.40**	12.57*	13.57NS	21.77*
	SF	38.1	59.2	89.1	123.8	182.9	284.0	408.8	527.0	720.0	270.30						
	Mean	37.4	56.3	89.1	119.4	174.2	255.8	369.7	485.7	659.3	249.63						
RIR×*Desi*	RF	25.7	59.1	89.8	130.0	215.0	300.0	372.5	465.0	610.0	251.91						
	SF	28.8	60.8	95.0	132.5	200.0	260.0	315.0	427.5	560.0	231.07						
	Mean	27.3	60.0	92.4	131.3	207.5	280.0	343.8	446.3	585.0	241.49						
Desi×Fay	RF	29.4	46.3	75.1	99.4	172.0	227.0	342.0	429.0	528.0	216.47						
	SF	29.9	49.0	80.9	101.5	188.3	260.0	383.3	499.0	680.0	252.44						
	Mean	29.7	47.6	78.0	100.4	180.2	243.5	362.7	464.0	604.0	234.46						

[+]NS, P>0.05; *, P<0.05; **, P<0.01; [$]RF, rapid feather; SF, slow feather.

Table 3: Cloacal temperature (^{0}C) of rapid and slow feathered crossbred progeny

Genotype (G)	Feathering pattern ($FP^{\$}$)	Age (A) in days										LSD (SED) and significance level⁺					
		1	12	21	35	49	63	77	91	105	Mean	G	FP	A	G×FP	G×A	FP×A
Desi×RIR	RF	-	43.4	41.0	41.3	41.0	41.8	45.5	42.1	42.0	42.26	0.20**	0.24**	0.20**	0.17NS	0.35NS	0.28NS
	SF	-	50.5	41.0	41.2	40.9	42.1	42.0	42.0	42.2	42.76						
	Mean	-	46.9	41.0	41.3	41.0	41.9	43.7	42.1	42.1	42.51						
RIR×*Desi*	RF	-	40.2	41.3	41.7	41.6	36.7	43.2	42.2	42.2	41.13						
	SF	-	40.0	41.0	41.8	36.7	31.1	42.2	42.2	42.2	39.65						
	Mean	-	40.1	41.1	41.8	39.1	33.9	42.7	42.2	42.2	40.39						
Desi×Fay	RF	-	40.6	39.6	41.5	41.9	42.1	42.2	42.1	41.9	41.49						
	SF	-	40.5	41.0	41.6	41.8	42.0	42.2	42.1	42.1	41.66						
	Mean	-	40.5	40.3	40.6	41.9	42.0	42.2	42.1	42.0	41.58						

⁺NS, P>0.05; *, P<0.05; **, P<0.01; $RF, rapid feather; SF, slow feather.

Table 4: Relationship of live weight with cloacal temperature among genotypes and feathering pattern

Parameter	Dependent variable	Independent variable	a	aSE	b	bSE	t	r
Genotype								
Desi×RIR	Weight (g)	Cloacal temp (°C)	29.30	27.873	0.047**	0.008	6.171	0.43**
RIR×*Desi*	Weight (g)	Cloacal temp (°C)	17.62	39.865	0.044**	0.011	4.026	0.46**
Desi×Fayoumi	Weight (g)	Cloacal temp (°C)	24.77	16.469	0.032**	0.005	6.828	0.36**
Feathering pattern								
Rapid	Weight (g)	Cloacal temp (°C)	25.20	16.403	0.036**	0.005	7.704	0.42**
Slow	Weight (g)	Cloacal temp (°C)	24.66	23.474	0.044**	0.007	6.737	0.42**

a= intercept; aSE= standard error of intercept; b= regression co-efficient, bSE= standard error of regression co-efficient; t= student's t value; r= correlation co-efficient.

Table 5: Carcass characteristics of among genotypes and feathering pattern

Parameter	FP#	Genotype (G)			Mean	SED and significance level		
		Desi×RIR	RIR×*Desi*	*Desi*×Fay		G	FP	G×FP

Dressing weight (%)	RF	47.32	48.71	43.68	46.57	4.733^{NS}	3.869^{NS}	6.693^{NS}
	SF	59.12	53.20	53.88	55.40			
	Mean	53.22	50.96	48.78	50.99			
Total meat yield (%)	RF	20.56	22.85	18.33	20.58	1.967*	2.435**	2.782*
	SF	28.69	25.47	24.55	26.24			
	Mean	24.63	24.16	21.44	23.41			
Breast meat yield (%)	RF	6.96	7.69	6.52	7.06	0.210^{NS}	0.634**	1.101**
	SF	9.91	8.57	9.02	9.17			
	Mean	8.44	8.13	7.77	8.12			
Thigh meat yield (%)	RF	6.01	6.69	5.32	6.01	0.615**	0.500**	0.871**
	SF	9.26	7.65	8.05	8.32			
	Mean	7.64	7.17	6.69	7.17			
Drumstick meat yield (%)	RF	4.90	5.41	4.12	4.81	0.406*	0.500**	0.575*
	SF	6.07	5.68	5.89	5.88			
	Mean	5.49	5.55	5.01	5.35			

#Feather pattern; RF = rapid feathered, SF = slow feathered; NS = not significant (P>0.05); * = significant (P<0.05); **= highly significant (P<0.001).