

ANALYSIS OF GENETIC DIVERSITY FOR SOME GENETIC GROUPS AND COMPARING THEIR PRODUCTIVE PERFORMANCE IN CHICKENS

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SUMMARY

In the current study; genetic diversity and productive performance were estimated for some genetic groups: normally feathered (*nanaff*), frizzled feather (*nanaFf*), naked neck (*Nanaff*) and naked neck frizzled feather (*NanaFf*) ones. It seems that both *Na* and *F* genes play an important role in improving some economical traits such as: weight gain, feed conversion, live body weight, breast muscle percentage and edible parts percentage. The corresponding values for these traits were (598.65gm), (3.70), (1389.19gm), (11.14%) and (73.39%) for naked neck genotype (*Nanaff*), while it was (498.75gm), (3.90), (1315.42gm), (10.23%) and (72.80%) for *nanaFf* and (512.22gm), (3.73), (1410.21gm), (12.02%) and (73.83%) for naked neck with frizzled feather genotypes *NanaFf* ones when the comparison held with their normally feathered counterparts. Moreover; the naked neck (*Na*) gene decreased significantly abdominal fat percentage 0.48% for the *Nanaff* and 0.41 % for *NanaFf* compared to normal feather (*nanaff*) genotype. The same trend was noticed for frizzled genotype, but it had a slight effect (0.09). Both *Na* and *F* genes improved meat production under the condition of this study. The similarity between genetic differences in this experiment appeared to be 80%, 70%, 90 and 70% for *nanaff*, *Nanaff*, *nanaFf* and *NanaFf* respectively. The degree of diversity or similarity within different genotypes reflected purity or identity of these categories. But, it is necessary to keep some variations between and within genotypes or breed to avoid the disadvantage of inbreeding.

Keywords: Chickens , carcass, frizzle, genetic diversity, naked neck

INTRODUCTION

In the recent years, climate changes led to an increase in the ambient temperature, resulting in misfortune economic losses. The high temperature causes high mortality percentage and also reduces some production traits in both broiler and laying stocks especially when the high temperature was accompanied with high relative humidity (Kgwatalala *et al.*, 2012 and Khalid *et al.*, 2012). It is well known that commercial strains, specialized in meat or egg production, were selected under environmental condition differed from that of hot climates. Their made commercial strains suffered from heat stress and their production of meat and egg was decreased. Commercial broiler strains suffered more from elevating temperature because these strains are heavy and have a high metabolic rate. The naked neck and frizzle genes are believed to improve adaptability in the tropics and hot climate, (Mahrous and Radwan 2011 and Liyanage *et al.*, 2015) by increasing heat dissipation through decrease feather coverage. Also, these genes had a positive effects in improving economic traits such as growth rate and meat yield under high or moderate ambient temperatures (Matthew *et al.*, 2011, Thutwa *et al.*, 2012, Kgwatalala *et al.*, 2013 and Dorji and Sunar, 2014). Ajayi *et al.* (2011); Rajkumar *et al.* (2011) and Radwan *et al.* (2015) studied the beneficial effects of naked neck and frizzled genes in improving the

productive traits and immunity competence under heat conditions.

In 1978, day old chicks carried the mutation naked neck gene and it was imported from France. Since, this naked neck gene was introduced into local strains mainly Fayoumi. While, Zein El-Dein *et al.* (1981) studied the effect of Naked neck gene in improving the productive performance of both imported and local strains under Egyptian conditions. Several studies since 1981 was achieved to verify the obtained results about the effect of this gene (*Na*) on improving growth rate and other productive traits. Radwan *et al.* (2015) measured immunity competence for these genotypes (normally feathered (*nanaff*), frizzled feather (*nanaFf*), naked neck (*Nanaff*) and naked neck frizzled feather (*NanaFf*) ones by using the specific microstallite studies and gene frequencies studies for egg production traits.

In the last few years, studies were more interested in using molecular genetics techniques in poultry breeding programs. These modern programs indicated a great importance in maintaining genetic variation within and between commercial lines and exotic populations. Also, RAPD and microstallites techniques were used in genomic mapping; identification of genes controlling productive traits and analyzing the degree of inbreeding; response to selection for stocks maintained under long-term selection programs and analyzing the genetic relationships among species at the molecular level

(Heyen *et al.*, 1999, Radwan 2014a&b, and Oni *et al.*, 2016). The present research was conducted to study the genetic diversity and some productive traits of four genetic groups.

MATERIALS AND METHODS

Mating and management

This experiment was carried out at the poultry breeding farm, Faculty of Agriculture, Ain Shams University. The artificial insemination procedure was used to inseminate normally feathered females (nanaff) with the semen of heterozygous naked neck frizzled males (NanaFf). The obtained offspring was of four genetic groups; normally feathered (nanaff), frizzle feather (nanaFf), naked neck (Nanaff) and naked neck frizzled feather (NanaFf). The naked neck and the frizzle genes are autosomal genes. The naked neck gene is an incompletely dominant one. It is easy to differentiate between homozygous and heterozygous genotypes by a tuft of feathers on the ventral side of the neck above the crop. The homozygous (NaNa) genotype, either lack this tuft or it is reduced to a just few pin feathers or small feathers. While, homozygous frizzled birds, the edges of all feathers are extremely curved. These feathers are easily broken and therefore, the birds appear quite bare. The unmodified heterozygotes have the feather shafts and barbs of contour feathers curved, to a much lower extent than the homozygote (Fathi *et al.*, 2013).

These genetic groups were winging-banded and brooded in electrical brooding batteries from hatching time and up to the end of the experiment (16 weeks of age) and were reared under similar

environmental conditions. During the experimental period the degree of the recorded temperature ranged from 28.7°C to 32.5°C.

The studied traits

Feed conservation and weight gain were recorded from the 8th to 12th weeks of age for males only from different groups. At the 12th weeks of age, two hundred eighty males from all genotypes, 70 males from each genotype group used in this study were slaughtered after recording their live individual body weight. After dressing, the slaughtered birds were weighed and all the inedible parts were removed after recording their weights (intestines, proventriculus, gall bladder, spleen, esophagus and crop) and their percentage were calculated also. Giblets (heart, liver, gizzard) were cleaned and weighted to calculate their percentages. Abdominal fat was removed, weighted and its percentage was calculated. The breast muscles were weighed and their percentages were calculated.

DNA extraction and purification

Blood samples were collected, in EDTA tubes, from 10 individual males chosen randomly from each genotype group (nanaff, nanaFf, Nanaff and NanaFf). Then, these blood samples of each genotype group were mixed well. After this 3 samples from each mixture were taken to extract DNA (Bulk) by using Axygen Scientific kit, inc. USA Cat. No. AP-MN-BL-GDNA-50. The primers used are listed in table 1. DNA were extracted for each genotype and grouped as follows: 1,2 and 3 for nanaff; 4,5 and 6 for Nanaff; 7,8 and 9 for nanaFf and 10,11 and 12 for NanaFf.

Table 1. Primer name, sequence and annealing temperature

Primer name	Primer sequence	Annealing temp.
ADL 115	F (5' GGATGAGAAGAAAGGCA 3')	50° C
	R (5' CAATGGTGGTTCAGGTAATC 3')	56° C
ADL 209	F (5' GGTTAGCTCCCTCCTTCCAG 3')	63° C
	R (5' AAGGAAACAAAGAGAAATCC 3')	52° C
ADL 231	F (5' ACTATTAGCCTGGGGAGAGC 3')	60° C
	R (5' TCACTCCAGCTTGAGACAGG 3')	60° C
APH 23	F (5' GGATGAGAAGAAGAAAGGCA 3')	56° C
	R (5' AAGGAAACAAAGAGAAATCC 3')	52° C
APH 24	F (5' GGATGAGAAGAAGAAAGGCA3')	56° C
	R (5' CAATGGTGGTTCAGGTAATC 3')	56° C
A-03	5' AGTCAGCCA 3'	32° C
B-03	5' CATCCCCCTG 3'	34° C
G-03	5' GAGCCCTCCA 3'	34° C
Z-03	5' CAGCACCGCA 3'	34° C

Polymerase Chain Reaction (PCR) and amplification condition:

PCR was performed in 25 µl volumes containing 12 µl of PCR Master mix (ROVALAB 2x Red PCR

Master Mix, Kantstr, Germany), 2 µl of primer (10 pmol/µl), 1 µl genomic DNA (50 ng/µl) and 10 µl sterile deionized water were added. Amplification was performed in a thermo cycler (Long Gene-

MG96G/china) with the following temperature profiles: initial denaturation 95°C for 4 min, 35 cycles (denaturation 95°C 1 min/ annealing temp depending on the primer for 1 min/extension 72°C 1 min) and Final extension 72°C for 5 min. The reaction was held at 4°C.

Statistical analysis:

One-way analysis of variance was performed with a genotype effect. The statistical model could be described as follows: $Y_{ij} = \mu + G_i + e_{ij}$.

Where; μ = overall mean, G_i = genotypes effect and e_{ij} = experimental error.

According to the General Linear Models (GLM) Procedure of SAS User's Guide, 2013.

Analysis of the amplified DNA:

The PCR product (15 μ l) was resolved by electrophoresis by using 2 % agarose gel (supplemented with ethidium bromide) at 70 V for 90 min. DNA ladder (100 bp Larova GmbH / Germany) was used as DNA size markers. Gels were visualized with UV light and photographed by a Sony digital camera.

Similarity, diversity and relationships between genotypes were organized based on the similarity matrix established with neighbor joining (NJ) method using Jaccard formula $d_{jk} = M/(M+N)$. Genetic similarity among chicken groups was estimated by scoring the presence and absence of bands produced by the primers. The presence (1) or absence (0) of a band of a particular molecular weight was scored as two alleles at a single locus. The NTSYSpc 2.01 software package was using present relationships between genotypes as dendrogram (Rohlf, 1998).

Eventually, calculated percent polymorphism by using equation follow:

$$\text{Percent polymorphism} = \frac{\text{Total number of polymorphic bands}}{\text{Total number of bands}} \times 100$$

RESULTS AND DISCUSSION

Feed conversion ratio and body weight gain of male chickens from different studied genotypes (nanaff, nanaFf, Nanaff, NanaFf) are shown in table 2. The naked neck gene (Nanaff) significantly increased weight gain by about 598.65 gm; frizzle gene (nanaFf) by about 498.75gm and naked neck frizzle feather by about 512.22 gm compared to normal feather counter parts. On the other hand; the feed conservation was improved by about 0.25 (Nanaff); 0.05 (nanaFf) and 0.22 (NanaFf) when compared to normal feathers. The gene responsible for naked neck reduces the feather cover of birds and improve their heat tolerance and heat loss by radiation. This reflects on improving feed conversion under high temperature. As for, frizzle gene, it modifies the feather growth structure and this leads to curve outward cause facilitated bombard feather reason lower plumage coverage (Haaren-Kiso *et al.*, 1994). Alabi *et al.* (2012) and Hossain *et al.* (2012) recorded that naked neck gene improved thermoregulation, feed consumption, feed conservation and decrease body temperature under high (30°C) or moderate (22°C) ambient temperatures. Naked neck gene significantly improved feed conservation and body weight gain than the frizzle gene.

Table 2. Mean of feed conversion ratio and body weight gain of male birds from different studied genotypes (nanaff, nanaFf, Nanaff, NanaFf)

Traits	Genotype				SEM	Prob
	nanaff	nanaFf	Nanaff	NanaFf		
Feed coverion (8-12) weeks	3.95 ^b	3.90 ^b	3.70 ^a	3.73 ^a	0.14	0.03
Body weight gain (8-12) weeks	457.82 ^c	498.75 ^b	598.65 ^a	512.22 ^{ab}	40.12	0.01

^a, ^b and ^c Means within the same row, with different subscripts are significantly different (P < 0.05), NS = not significant

Carcass characteristics of male chickens are listed in table (3) for the different genetic groups handled in this study. It should be noted that both genes (Na and F) caused a significant increase in live body weight, carcass percentage, breast muscle percentage and edible parts percentage, the corresponding value were (1389.19gm), (69.02%), (11.14%) and (73.39%) for Nanaff genotype and (1315.42gm), (68.35%), (10.23%) and (72.80%) for nanaFf genotype and (1410.21gm), (69.36%), (12.02%) and (73.83%) for NanaFf genotype, respectively when compared to normal feather (nanaff) ones.

The naked neck gene (Nana) decreased plumage by about 20 % (Mérat, 1990). This may be reflected on reducing the requirements from proteins. It may

be directed to breast muscle growth and causing a significant increase in these muscles instead of plumage growth. So, it could be postulated that the naked neck gene plays an important role in increasing protein deposition in breast region and causing heavier breast muscles weights than that of the normal gene (REF).

Moreover; the naked neck gene significantly decreased abdominal fat percentage by 0.48 as shown in table (3). The same trend exist on the NanaFf genotype (0.41). While, the effect of F gene alone on the same traits was very slight (it was 0.82%) for nanaFf genotype and (0.91%) for the nanaff genotype. The abdominal fat is considered an undesirable trait that decreases meat quality (Berri *et*

al., 2005). Thus broiler strains are selected for reduced abdominal fat weight. The decrease in the abdominal fat, due to the naked neck gene, may be

because of the energy for regulating body temperature for the limited feather covered.

Table 3. Mean of carcass characteristics of male chickens as affected by naked neck (Na), frizzled (F) and double segregation genes

Traits	Genotype				SEM	Prob
	nanaff	nanaFf	Nanaff	NanaFf		
Live body weight (gm)	1297.86 ^b	1315.42 ^b	1389.19 ^{ab}	1410.21 ^a	90.12	0.0 ^r
Carcass (%)	65.89 ^b	68.35 ^a	69.02 ^a	69.36 ^a	1.36	0.01
Breast muscle (%)	10.01 ^c	10.23 ^{ab}	11.14 ^b	12.02 ^a	1.01	0.001
Abdominal fat (%)	0.91 ^a	0.82 ^a	0.48 ^b	0.41 ^b	0.02	0.02
Heart (%)	0.55	0.56	0.56	0.55	0.02	NS
Liver (%)	1.85	1.93	1.89	1.91	0.14	NS
Gizzard (%)	1.81	1.96	1.92	2.01	0.10	NS
Giblets (%)	4.21	4.45	4.37	4.47	0.12	NS
Edible parts (%)	70.10 ^c	72.80 ^b	73.39 ^a	73.83 ^a	1.86	0.01
Inedible parts (%)	29.90 ^a	27.20 ^{ab}	26.61 ^b	26.17 ^b	2.013	0.01

^a, ^b and ^c Means within the same row with different subscripts are significantly different ($P < 0.05$), NS = not significant.

Clarification of the population structure of the different studied genotypes (nanaff, , Nanaff, nanaFf, and NanaFf) are shown in Figs. (1, 2, 3, 4 and 5). Degree of similarity within nanaff genotype reached 100% in sample number 1. However, the similarities among this genotype (nanaff) reached approximately 95% and 90% in samples (2) and (3), respectively. While, degree of similarity within Nanaff genotype ranged from 60-70% as shown in Fig. (2). Also, Figs. (3 and 4) show that the degree of similarity reached 80% for nanaFf and NanaFf genotypes, respectively. The degree of similarity within different genotypes reflexes the degree of purity into these populations. A subdivision of the population was effected to relate

fixation of alleles compared to the total population (Hartl, 1998). On the other hand, the degree of similarity between and within the different genetic groups is shown in Fig. (5). It appears that the similarity between the different genetic groups were 80%, 70%, 90 and 70% for nanaff, Nanaff, nanaFf and NanaFf, respectively. Dorji and Sunar (2014) studied the relationship between Nigeria local breed, . which were posed the naked neck or frizzle genes and tried to classify them. As for the degree of similar they found that the normal plumage chickens occupied the first rank, followed by frizzled feather chickens while the naked neck or occupied by the latest order..

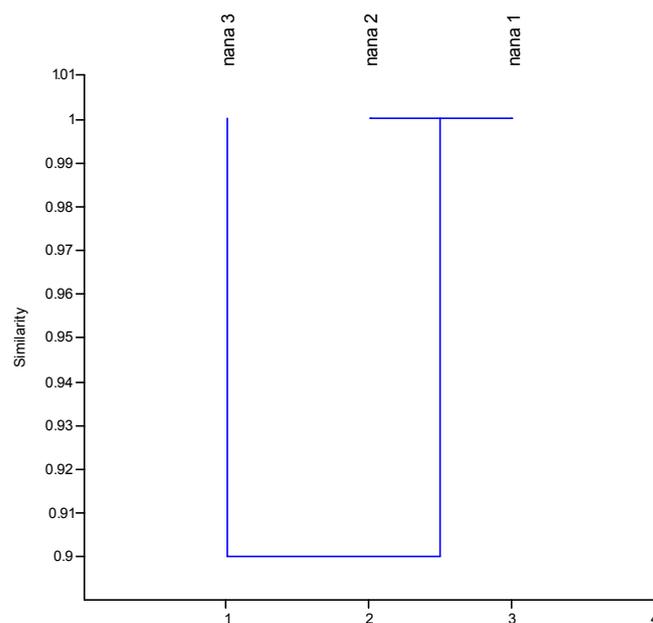


Fig.1. The degree of similarity within nanaff genotype. Blood samples collected from 10 males from nanaff genotype and mixed together, then 3 samples were taken dendrogram of similarity

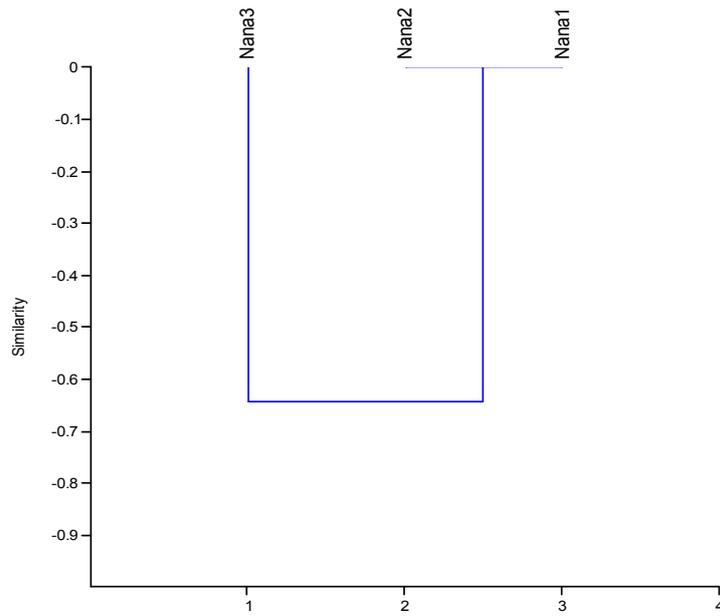


Fig. 2. The degree of similarity within Nanaff genotype. Blood samples collected from 10 males from Nanaff genotype and mixed together, then 3 samples were taken dendogram of similarty

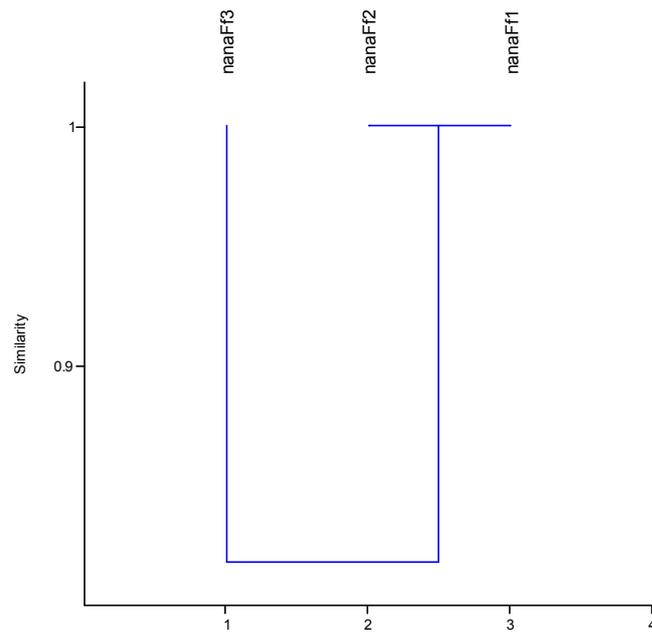


Fig. 3. The degree of similarity within nanaFf genotype. Blood samples collected from 10 males from nanaFf genotype and mixed together, then 3 samples were taken dendogram of similarty

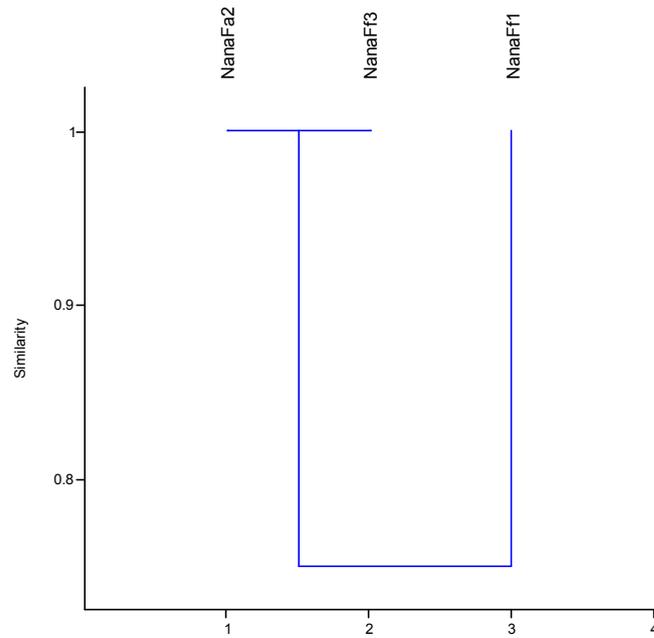


Fig .4. The degree of similarity within NanaFf genotype. Blood samples collected from 10 males from NanaFf genotype and mixed together, then 3 samples were taken dendrogram of similarity

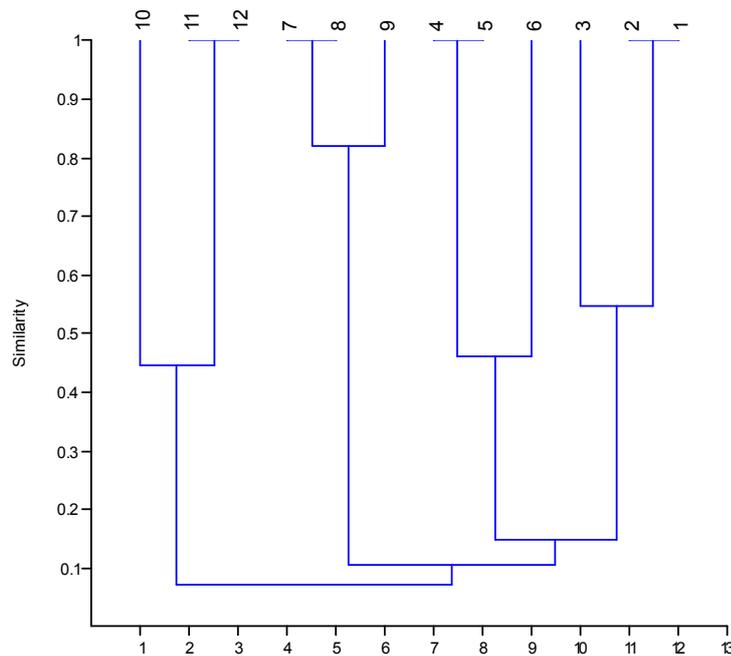


Fig. 5. Dendrogram the coefficient of genetic similarities between and within the different studied genotypes. Numbers 1,2 and 3 for genotypes nanaff, 4,5 and 6 for Nanaff; 7,8 and 9 for nanaFf and 10,11 and 12 for NanaFf

Although, the four genetic groups originated from the same genetic origin (normally feathered females (nanaff) with the semen of heterozygous naked neck frizzled males (NanaFf)), the similarity within each genetic group reached 100%, and that means that the diversity appears between genetic groups. Peters *et al.* (1998) achieved the same results on Nigeria local strains which carried the same genes (naked neck and

frizzled). Olawumi and Ogunlade (2009) and Sola *et al.* (2013) stated that to achieve genetic improvement in Nigeria local strain, the first step should be studying its genetic diversity.

Polymorphism information for different genotypes provides an estimate of the similarity or diversity. However, we must keep it in mind to avoid the disadvantages of inbreeding.

CONCLUSION

The naked neck gene had a great benefit in improving meat production, due to increasing breast muscle percentage and decreasing abdominal fat percent. Moreover, the naked neck gene improves heat tolerance of birds. The frizzled gene is playing a complementary role to increase acclimatization under the high heat circumstance. Also, it maximizes improvement in meat product. The degree of similarity or diversity within different genotypes reflects purity or identity of those genotypes. Although it must keep some variation within genotypes to avoid the disadvantage of inbreeding.

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تحليل التنوع الوراثي ومقارنة الاداء الانتاجي لبعض المجموعات الوراثية في الدجاج.

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تم دراسة التنوع الوراثي وقياس الاداء الانتاجي وتقديرها لبعض المجموعات الوراثية في الدجاج (طبيعية التريش - مجمدة التريش - عرى الرقبة - عرى الرقبة ومجمدة التريش حيث اظهرت النتائج الدور الهام التي تلعبه كلا من العوامل الوراثية (عامل عرى الرقبة - عامل مجمد التريش) في تحسين الصفات الاقتصادية (الانتاجية) ومنها على سبيل المثال (الزيادة الوزنية - معامل التحويل الغذائي - وزن الجسم الحي - عضلات الصدر- الجزء المأكول من الذبيحة) وكانت النتائج المتحصل والقيم المقابلة لهذه الصفات كالآتي:- ٥٩٨.٦٥ جم - ٣.٧ - ١٣٨٩.١٩ جم - ١١.١٤% وايضا ٧٣.٣٩% للعامل الوراثي عرى الرقبة ذو للتركيب الوراثي *Nanaff* بينما العامل الوراثي الريش المجمد ذو للتركيب الوراثي *nanaFf* كانت النسب كالتالي ٤٩٨.٧٥ جم- ٣.٩ - ١٣١٥.٤٢ جم- ١٠.٢٣% وايضا ٧٢.٨٠% على التوالي. وكذلك ايضا تم ملاحظة تقليل نسبة دهن البطن للدجاج عارى الرقبة ومجمدة التريش *NanaFf* ٤٨.٠% و ٤١.٠% بالمقارنة لدجاجات طبيعية التريش وعلى نفس الاتجاه وعلى نفس للاتجاه تم ملاحظة انخفاض ضئيل بنسبة ٠.٠٩% بالنسبة للعامل الوراثي الريش المجمد بمقارنة بدجاج طبيعي التريش. كما تم ملاحظة تحسين في انتاجية اللحم للدجاج الحامل للعامل الوراثي عرى الرقبة والعامل الريش المجمد فتكذلك الدجاج الحامل للعاملين معا. بينما سجلت نسبة التشابه الوراثي داخل المجموعات الوراثية وكانت كالتالي ٨٠%- ٧٠%- ٩٠%- وايضا ٧٠% بالنسبة لطبيعي التريش وعارى الرقبة ومجمدة التريش وايضا عارى الرقبة ومجمدة التريش على التوالي. وترجع اهمية تقدير نسبة التشابه والاختلاف داخل المجموعات الوراثية الى انها تشير لدرجة النقاوة والاختلاف بين الافراد كما انه يجب الحفاظ على وجود الاختلاف (التنوع) بين وداخل المجموعات الوراثية تعمل على عدم حدوث تربية داخلية وتساعد على اجراء عمليات التحسين داخل المجموعات الوراثية.