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Polymorphism of Microsatellite markers and Their Association with Egg Production Traits in Iraqi Chickens

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Abstract

The present study was conducted on local Iraqi chickens and two strains of commercial laying hens (ISA Brawn and Ross Hen) as control. To estimate genetic Diversity using microsatellites and their association with egg production traits, three microsatellites markers, located on 1, 5 and E5C10 chromosomes were used in pesent study. A total of 100 varieties of three chicken populations were genotyped for three microsatellite markers by polymerase chain reaction (PCR) to evaluate the genetic Diversity (GD) among populations using Nei Index similarity mean.

The present study show that local chicken populations were more diverse than control populations. Genetic Diversity among populations was obtained using Nei Index similarity mean. The present results indicate that the Highest GD among local chickens (0.82) and the lowest GD (0.4) and when comper local chickens with control strains found that the highest GD was (0.76) when comper with ISA Brawn, and (0.702) when comper the study population with Ross Hen strain and the lowest GD was (0.673) when comper with ISA Brawn strain, (0.661) when comper the study population with Ross Hen strain, that's mean the Three microsatellite genetic markers applied in the present study success to reveal high degree of similarity among the three population used here. The genetic distance revealed that local chickens are mostly related to ISA Brawn strain more than Ross Hen strain.

Key words: Genetic diversity, SSR markers, microsatellite marker, Iraqi chickens, poultry microsatellite, globule strains Layer chickens, Nei Index

الخلاصة

أجريت الدراسة الحالية لغرض تحديد التنوع الجيني للدجاج العراقي المحلي مقارنة مع اثنتان من السلالات التجارية العالمية للدجاج البياض (سلالة ISA Brawn و سلالة Ross Hen) كسيطرة باستخدام التوابع الكروموسومية الدقيقة microsatellite و علاقتها مع صفات إنتاج البيض و تم استخدام ثلاثة من التوابع الكروموسومية للكروموسومات 1، 5، E5C10. تضمنت الدراسة 100 عينة من المجتمعات الثلاثة للدواجن و استخدام التوابع الكروموسومية الدقيقة microsatellite لتحديد التنوع الجيني بوساطة تفاعل البلمرة التسلسلي Polymerase Chain Reaction (PCR) للتوابع الكروموسومية. وجد أثناء الدراسة إن مجتمع الدواجن المحلية أكثر اختلافا بالمقارنة مع مجتمعي سلالتي السيطرة. تم قياس الاختلاف الجيني (GD) بين المجتمعات باستخدام دليل Nei للتماثل الجيني . اشارت النتائج الحالية بان أعلى اختلاف جيني (GD) بين مجتمع الدواجن المحلية كان بمقدار (0.82) و اقل اختلاف جيني كان بمقدار (0.4) و عند مقارنة الدواجن المحلية بسلالات السيطرة وجد ان أعلى (GD) كان (0.861) عند مقارنة مجموعة الدراسة مع ISA Brawn و كان (GD) (0.867) عند مقارنة مجموعة الدراسة مع سلالة Ross Hen و أوطئ GD (0.673) عند مقارنة مجموعة الدراسة مع سلالة ISA Brawn و كان GD (0.661) عند مقارنة مجموعة الدراسة مع سلالة Ross Hen، و هذا يعني إن نتائج التوابع الكروموسومية الدقيقة microsatellites الثلاثة المستخدمة في هذه الدراسة كانت ناجحة في الحصول على أعلى درجة تقارب (تماثل) بين المجتمعات الثلاثة المستخدمة في هذه الدراسة.

الكلمات المفتاحية: التوابع الكروموسومية، الاختلاف الجيني، الدجاج العراقي، التوابع الكروموسومية للدواجن، الدجاج البياض، سلالات عالمية للدجاج البياض.

Introduction

local chickens in Iraqi are valuable genetic resources due to their adaptability to difficult condition when raised in pastoral area or when reared in outer system as free range chickens, these chickens responded well to improve their environment conditions, especially, nutrition and exhibited improvement in body weight at sexual maturity and egg weight and also found that they classified as a layer type especially one type of them (Brown line) showed a good performance for egg production among other lines (Brown, Barred, Black, White, White neck-naked and Brown neck-naked). (Razuki and AL-Shaheen 2011).

Species viability depends on stochastic and deterministic demographic, environmental and genetic factors. Estimations of genetic diversity can be very important in programs for the conservation of biodiversity but such data need to be used with caution. In some situations, such as when the habitat is being rapidly destroyed, it is futile to be concerned with long-term goals such as genetic variability analysis (Haig, 1998) and it would be better to concentrate resources on the effective protection of the environment instead of conducting genetic studies. However, genetic diversity is important for the population to be able to face future environmental changes and to ensure a long term response to selection (Faria, 2006)

Neutral genetic markers are assumed to reflect adaptive genetic variation that is important to the evolutionary potential of the species (Hunter, 1996; Frankham *et al.*, 2002) and consequently, the selection of useful molecular markers is necessary to conduct these studies. Among the molecular techniques available, DNA fingerprinting, developed by Jeffreys *et al.* (1985), has been widely utilized in studies of various groups of animals including threatened species of birds (Miyaki *et al.*, 1993; Craveiro and Miyaki, 2000; Caparroz *et al.*, 2001). This technique is based on the detection of Microsatellites which are VNTRs, but are less frequent in the avian genome than in other organisms (Primmer *et al.*, 1997). This kind of marker has the advantage of being able to be amplified by PCR (and thus, it does not require large amounts of DNA), is usually highly polymorphic in the number of repeat units and shows a single locus pattern which allows the comparison of populations based on their allele frequencies (Bruford *et al.*, 1996). However, the development of microsatellite markers also requires a large amount of work, but, fortunately, many primers developed for one species can be used in related species (Moore *et al.*, 1991; Crooijmans *et al.*, 1993; Hanotte *et al.*, 1994; Bruford *et al.*, 1996; Primmer *et al.*, 1996). Microsatellites have been useful in many animal conservation studies, including Komodo dragons (Ciofi and Bruford, 1999), turtles (Fitzsimmons *et al.*, 1997), whales (Buchanan *et al.*, 1996), wolves (Roy *et al.*, 1994), snakes (Prosser *et al.*, 1999), bears (Paetkau *et al.*, 1998), butterflies (Keyghobadi *et al.*, 1999) and birds (Hansson *et al.*, 2003; Nesje *et al.*, 2000; Johnson *et al.*, 2003). Another approach for estimating genetic variability is to use primers of short repeated sequences (inter simple sequence repeats, ISSRs) to amplify anonymous genomic regions between two microsatellite loci. The principal advantage of this kind of approach is that it is not necessary to construct genomic libraries (Faria, 2006). One of the most important uses of molecular markers is to determine the genetic variability in species and populations that Which suffer from lack of offspring Printed in the new generations and an example of the Iraqi domestic chicken (AL- Hassani 2000) so come the aim of this study which was Attempt to get the local Iraqi hybrids optimum recipes to produce eggs, depending on the study of microsatellites compared to global strains such as ISA Brawn and Rose Hen strains.

Materials and Methods

Chicken populations: Where relevant throughout this study, chicken population, breed, line and strain will be referred to as population. One hundred chicken varieties were collected from the rural areas of Hilla .On the other hand, two global strains chickens were considered in this study; the ISA Brawn layer chicken and Ross Hen parent stock chicken.

Sample and DNA: Approximately 250 µl blood was collected from each bird and genomic DNA was isolated following "Distilled Water" Based Method for Genomic DNA Extraction from the Whole Blood of Mammals and Hens (Al-Shuhaib, 2015). The quantity and quality of DNA was evaluated on spectrophotometer and through 1.5% agarose gel electrophoresis

Microsatellites: Three microsatellites namely, ADL020, ADL023 ADL158 which were located on chromosome no. 1, 5 and E5C10 were considered (Table (1)) for the present study as these chromosomes harbor genes controlling growth, reproduction and disease resistance traits. Chromosome1 presents ADL020 while chromosome 5 carries ADL023 microsatellite and chromosome E5C10 presents microsatellite ADL136.

Table (1) Polymerase chain reaction (PCR)Microsatellite markers utilized

	Microsatellite Marker	Primer sequence (5` − 3`)	Chromosomal location	No. of alleles	Allele size range (bp)	PIC value	Annealing temperature (C^o)
1	ADL020	GCACTCAAAAGAAAACAAT TAGATAAAAATCCTTCCCTT	1	6	98, 100, 102, 108,112, & 116	0.656	55*
2	ADL023	CTTCTATCCTGGGCTTCTGA CCTGGCTGTGTATGTGTTGC	5	5	166, 170, 178, 182, & 194	0.712	61*
3	ADL136	TGTCAAGCCCATCGTATCAC CCACCTCCTTCTCCTGTTCA	E5C10	6	134, 138, 142, 150, 162 & 170	0.723	49**

***References annealing temp.**

**** Gradient annealing Temp.**

Polymerase chain reaction (PCR): PCR was performed in 20 µl reaction mixture containing 100-200ng DNA template, 20 pM of each primer, 200 µM each dNTP, 1U Taq DNA polymerase and optimised quantity of $MgCl_2$. The optimum annealing temperatures which gave the best amplification has been presented in (Table (1)).

Poly acrylamide gel electrophoresis: Amplified products were electrophoresed on 40% nondenaturing polyacrylamide gel containing acrylamide and bis-acralymide in the ratio of 29:1. The gel was run at 200V for 45 min. in 1X TBE and stained with 0.1% silver nitrate following the standard protocol (Byun *et al.,* 2009).. The gel was visualized and documented under white light of gel documentation system.

Genotyping: Genotype of every animal was determined. Genotyping involved the recording of the homozygous or heterozygous state of the animal, as well as the size

of the respective alleles. The size of the allele was estimated by comparing with standard ladder DNA marker. Ultimately, the frequencies of different alleles were estimated in different breed groups following gene-counting method by Nei index genetic diversity.

Results

Genotypes: All the microsatellites were found to be polymorphic with the presence of three to 22 alleles in crossbred layer chicken populations (Table 1). Out of all the microsatellites, ADL020 and ADL136 were observed to be the highest polymorphic marker showing 22 alleles distributing over the lines. The Genotype frequencies for ADL023 microsatellite was varied from 0.46 to 0.78

Genetic Diversity: Genotypic diversity across all estimated parameters was observed in every population according to Nei genetic similarity equation (1972) standard genetic Diversity (GD):

$$S = \frac{2N_{xy}}{N_x + N_y}$$

The DNA bands generated by each primer were counted and their molecular sizes were compared with those of the ladder marker. The bands scored from DNA profiles generated by each primer were pooled together. Then the presence or absence of each DNA band was treated as a binary character in a data matrix to calculate genetic similarity. Mathematically, similarity coefficients or band sharing is shown in Tables (2 - 3).

Table (2) Genetic Diversity (GD)* between 15 individuals of local chickens by SSR

Microsatellite marker	Genotypes	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
ADL 020	1		0.418	0.573	0.502	0.484	0.572	0.526	0.436	0.492	0.49	0.472	0.559	0.625	0.771?	0.524
ADL 023	2	0.418		0.573	0.502	0.484	0.572	0.526	0.436	0.492	0.49	0.472	0.559	0.625	0.771?	0.524
ADL 136	3	0.738	0.465		0.542	0.685	0.47	0.684	0.526	0.568	0.64	0.64	0.488	0.59	0.61	0.599
	4	0.578	0.821?	0.67		0.526	0.572	0.637	0.784	0.816	0.315	0.8	0.601	0.716	0.426	0.711

ADL 020	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
ADL 023	0.494		0.48	0.478	0.598	0.4?	0.444	0.528	0.458	0.44	0.422	0.4?	0.466	0.405	0.516
ADL 136	0.639	0.566	0.583	0.769	0.638	0.46?	0.583	0.686	0.646	0.61	0.788?	0.585	0.61	0.688	0.641
	0.604	0.707	0.61	0.638	0.712	0.476?	0.677	0.529	0.631	0.499	0.666	0.537	0.657	0.72	0.74

*(GD) ≤ 1

Lowest similarity mean

Highest similarity mean

Table (3): Genetic Diversity (GD)* matrix among three different strains estimated according to Nei index similarity for microsatellite markers

Microsatellite markers	Strains	Local chickens (GD)*	ISA Brawn (GD)*	Ross Hen (GD)*
ADL020	Local chickens	0.5937	0.760	0.691
	ISA Brawn		0.861	0.867
	Ross Hen			0.804
ADL023	Local chickens	0.7041	0.703	0.661
	ISA Brawn		0.713	0.733
	Ross Hen			0.781
ADL136	Local chickens	0.749	0.673	0.702
	ISA Brawn		0.72	0.768
	Ross Hen			0.74

Lowest similarity mean

Highest similarity mean

Discussion

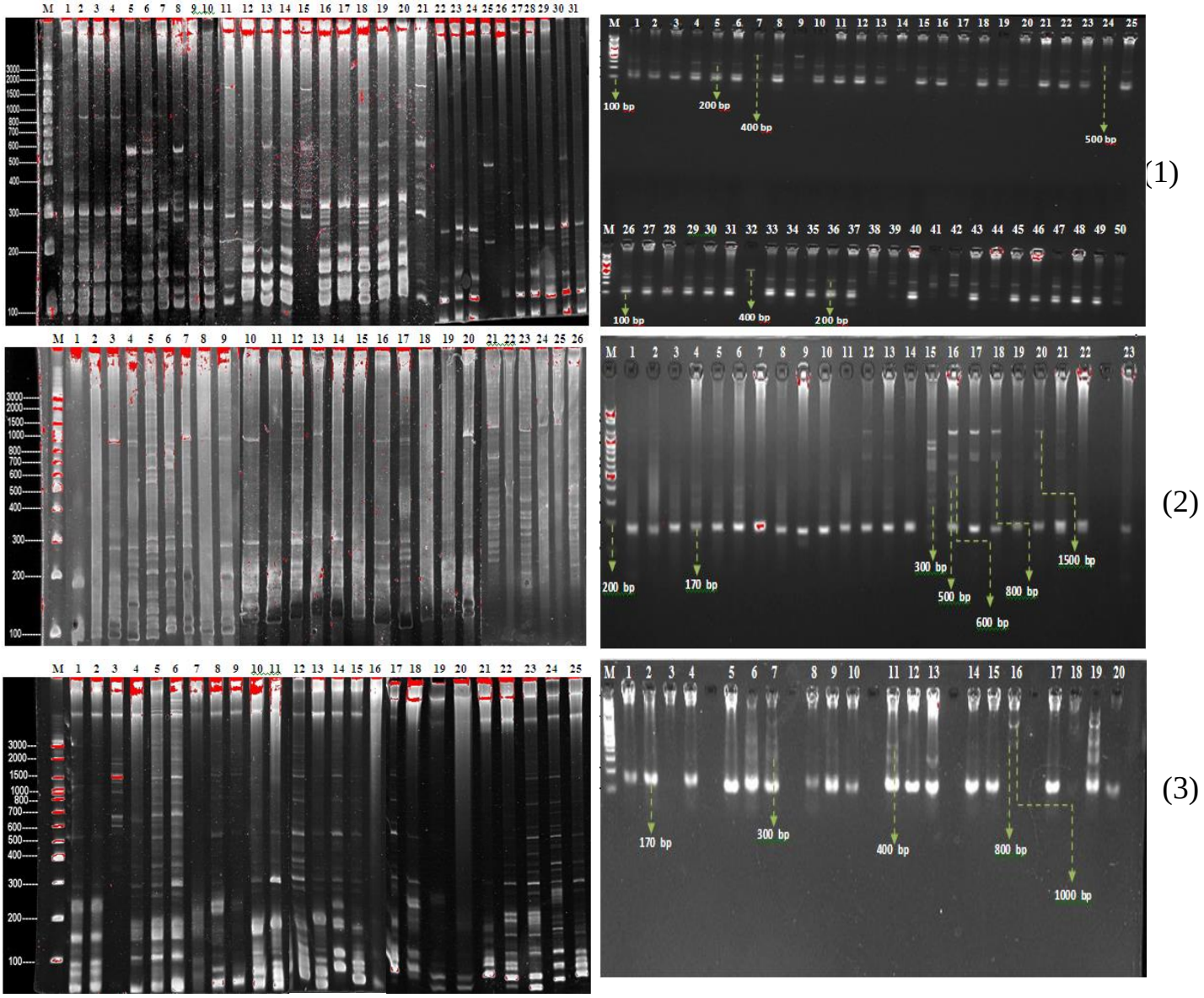
Genotypes: All the microsatellites were found to be polymorphic with the presence of three to 22 alleles in local chicken populations (Figure: (1)). Out of all the microsatellites, ADL020 and ADL136 were observed to be the highest polymorphic marker showing 22 alleles distributing over the lines. The Genotype frequencies for ADL020, ADL023 and ADL136 microsatellite were varied from 0.4 to 0.771, 0.46 to 0.788, 0.476 to 0.821, 0.456 to 0.799, 0.512 to 0.873 and 0.395 to 0.826, respectively among local chickens (Table 2). Our study revealed that the genotypic proportions were distributed from high to moderate whereas the allelic frequencies were ranging from 50 to 85%. . Osman *et al.* (2004) used microsatellites for studying genetic variability in the Oh-Shamo and its related chicken breeds emphasizing the potential of large genetic variability in birds (Chatterjee *et al.* 2008).

Genetic Diversity: The genetic Diversity among the three populations analyzed was described on the bases of genetic diversity (GD) (Table 3). The highest GD was recorded between local chicken and ISA Brawn strain (0.76) in ADL020 marker. However , The lowest GD was recorded between local chicken and Ross Hen strain (0.63) in ADL023 (Table 3). The ISA Brawn strain and Ross Hen strain were closer with maximum genetic similarity (0.804) in ADL020 marker and far with minimum GD (0.73) in ADL023 marker (Table 3). The genetic diversity values between different populations obtained in the present study were comparable with the values reported by earlier workers (Chatterjee *et al.* 2010).

In the present study the similarity among the locale Iraqi chickens and the Global strains are very high and this is very clear in Figure (2). The genetic diversity values ranging from (0.4 to 0.8), which indicate the substantial diversity (50% to 85%) among the varieties used for this study.

That's mean; Genetically There is a great convergence between the local chickens and the layer chickens.

(B) (A)



Figur (1): gel electrophoresis of Marker, microsatellite. M; DNA size marker other lanes local chickens, ISA Brawn and Ross Hen strains.

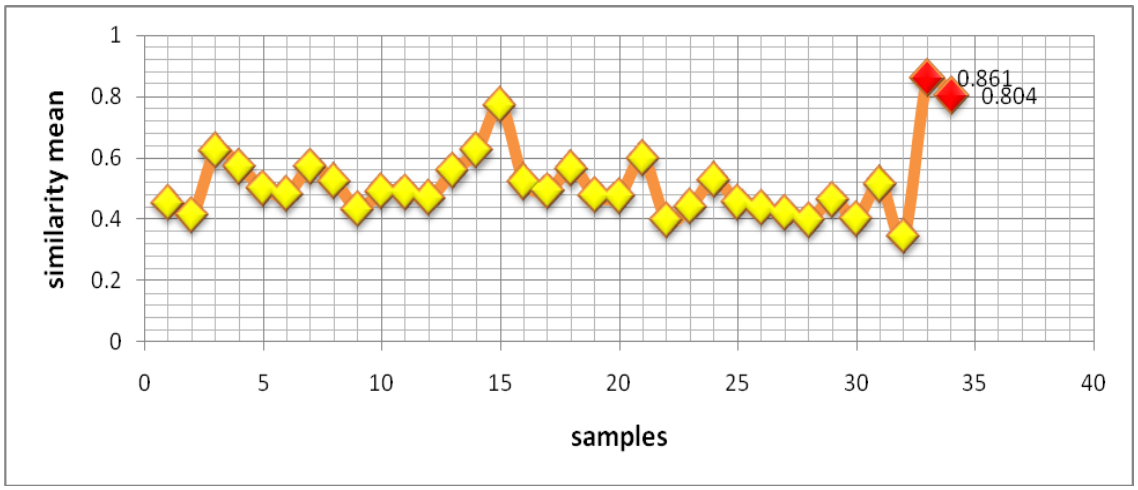
(A) Agarose gel electrophoresis of Marker, microsatellite.

Electrophoresis conditions: agarose concentration 1.5%, power applied: 135V (7V / cm), time of run: 45 min. staining method; precast ethidium bromide.

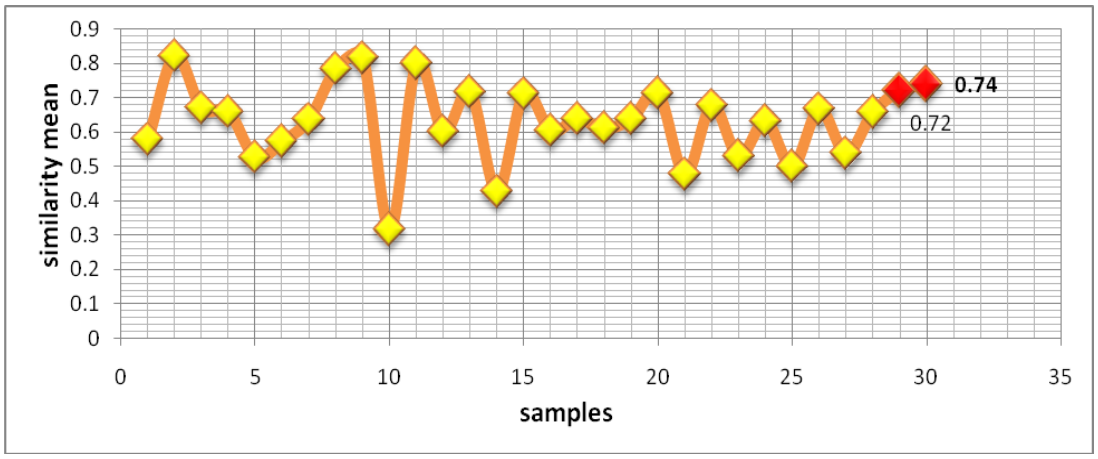
(B) Polyacrylamid electrophoresis of Marker, microsatellites.

Electrophoresis conditions: polyacrylamid concentration 40%, power applied: 200V (35mA / 6W), time of run: 45 min. staining method; post casting Page Gel red.

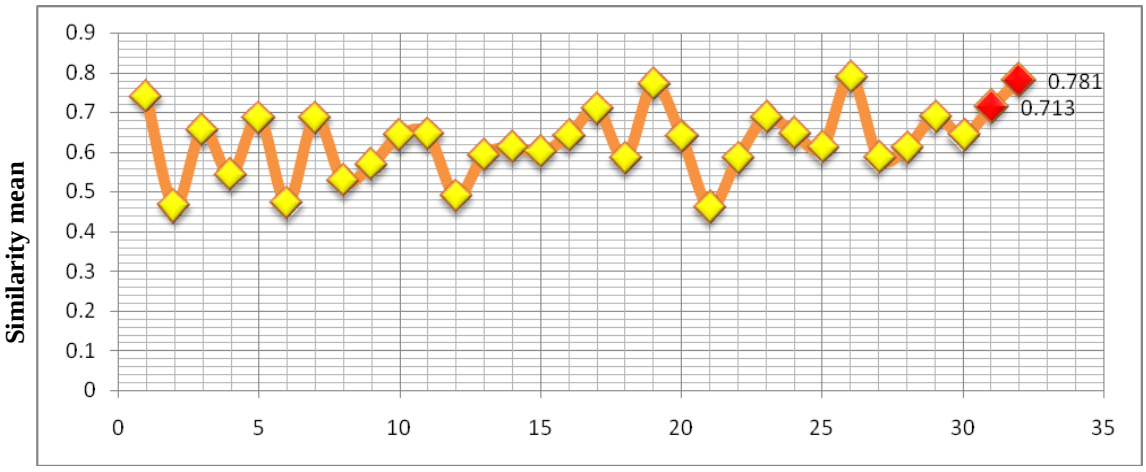
(1) ADL020, Microsatellite Marker
(2) ADL023, Microsatellite Marker
(3) ADL136, Microsatellite Marker



(A)



(B)



(C)

Figur (2): Summary of s mean of diversity $\{s=(2*N_{XY})/(N_X+ N_Y)\}$ for local chicken at (A) ADL020, (B) ADL136 and (C) ADL023 microsatellite marker and correlation with control breed

Local chicken mean
Control strain mean

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