Molecular and Morphological Characteristics of Aradi Goats with Prediction Equation of Growth Pattern

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Abstract

To specify morphological characteristics and choose preferable growth equation for kids up to 24 weeks of age, in Aradi goats, 338 kids of fathered by 25 sires and mothered by 153 does were used. Also, 74 individuals used in assisted with 29 microsatellites markers to identify molecular characterization. Results illustrated the linear equation was preferable according to easy compute, have two linear additive parameters and equation accuracy. The accuracy of that equation was very high ranged from 0.996 and 0.993 for predicted weights in male and female, respectively. Five loci showed only three alleles for each, while the rest of markers ranged from 13 alleles in loci CSRD247 and RM088 to four alleles in locus ILSTS011. The average of observed heterozygosity was 0.63 ranged 0.89 in BM2113 locus to 0.17 in ILSTS002 locus. The expected heterozygosity reached the maximum value in locus RM088 and the minimum one in locus OARE129, the average value was 0.65. The polymorphic information content values ranged from 0.85 (RM088) to 0.22 (OARE129). Thus, the investigation on a set of 29 microsatellites revealed high degree of genetic variability in Aradi goat indicating an important indigenous genetic resource.

Keywords: Microsatellites, Heterozygosity, Polymorphic information content

1. Introduction

Aradi is one of the most influential native goat breed in Saudi Arabia raised mainly for meat production, wide investigations were made to improve its productivity through crossing with exotic breed such as Damascus. As known, genetic improvement of any livestock breed depends on the identification of animals capable of transmitting their desirable characteristics to their offspring. For these reasons, deepness studies concerning on growth pattern with more precise techniques are highly needed. In this matter, mathematical functions of the animal growth curve have a wide interest (Morant & Gnanasakthy, 1989; Grossman et al., 1999; Mostert et al., 2003). Mathematical modelers seek to define parametric descriptors of growth shape. Many mathematical models have been used extensively to describe growth data in various species, Von Bertalanffy (Bertalanffy, 1957), Brody (Brody, 1945), Gompertz (Winsor, 1932), Logistic (Nelder 1961), Richards (Richards, 1959), Exponential Brody (Brody, 1945) simple and multiple regression (Leech & Healy, 1959; Sprint, 1967; Grizzle & Allen, 1969).

The most frequently important factors used to correct fixed effect are, season, sex, litter size at birth, age of dams, location and genetic groups. Brody (1945) used a mathematical function to describe a growth curve. As growth models are useful in growth prediction, linear equations have been used to express growth pattern at specific timer of age. Molecular characterization of any breed can be done by various methods and type of genetic markers. Microsatellites is one of the most important useful markers, intensively used in genomic variability and diversity studies (Ganai & Yadav, 2001; Kumar et al., 2005). These microsatellite markers considered to be of great benefits in analysis of paternity or genetic variability among and within studied breeds.

The present study was established to determine both molecular and morphological characteristics in Aradi goats in addition with formulate the prediction equation of growth pattern from birth to 24 weeks of age. Results obtained from these equations are very essential in decisions of culling or retaining a Goat at an earlier stage of life.

2. Materials and Methods

2.1 Growth Pattern

A total number of 338 kids fathered by 25 sires and mothered by 153 does belonging to Animal Experimental Farm, Qassim University, Saudi Arabia were utilized in this study. Body weights were measured at birth and every four weeks intervals thereafter up to 24 weeks of age. Linear equation with only two parameters was perfect fit to growth data on Aradi goat from birth to 24 weeks of age. The linear model $y_t = a + bt$ was convenient tendency of growth pattern, a biological mean of parameter (b) was average growth rate and (a) was initial body weight (Macciotta et al., 2011; Madalena et al., 1979; Kamidi, 2005). A total of 1533 records of Aradi kids growth weights were used to test the model. The fixed effect consider in this study were year of season which have 20 classes (4 seasons/year; five year from beginning of 2011 to the end of 2015, litter size at birth with 3 classes single, twins and triplet and sex with two classes male and female. The data was distributed through the traits shown in Table 1.

Table 1. Number of records per each trait, overall mean of the traits ± standard deviation (S.D), least square means of male and female ± stander error (S.E) per Kg

Trait	No.	Mean ± SD	LSMean ± SE	
			Male	Female
W0	338	3.01 ± 0.69	3.36 ± 0.06	3.06 ± 0.07
W4	257	7.03 ± 1.74	7.37 ± 0.18	6.78 ± 0.22
W8	229	10.23 ± 2.67	10.25 ± 0.26	9.28 ± 0.35
W12	224	13.42 ± 3.66	13.96 ± 0.38	12.34 ± 0.47
W16	189	16.25 ± 4.25	17.10 ± 0.53	14.88 ± 0.61
W20	171	18.32 ± 4.07	19.26 ± 0.55	16.60 ± 0.59
W24	151	21.50 ± 4.66	23.19 ± 0.63	19.66 ± 0.61

Model used:

$$Y_{ijkl} = \mu + YS_i + LSB_j + SX_k + e_{ijkl}$$

Where:

μ = the overall mean,

YS_i = the year season for i=1,…20,

LSB_j = the litter size effect at birth for j=1-3,

SX_k = the sex effect for k=1-2, and

e_{ijkl} = random error term

2.2 Application of Linear Model

The preliminary results of kids data showed that the linear function is preferable over the others and consequently appoint to use it. This conclusion was based on the coefficient of determination (R^2), finding biological interpretation of the estimated parameters. Hence this technique has its limitation, it is recommended to utilize for special stage intervals of growth. For the period from birth to 24 weeks of age, the linear equation fitted to the least square mean of seven continuous weights from birth (W0) and every 4 weeks according to model:

Model 1:

$$Y_t = a + bt + e_t \tag{1}$$

Where,

Y_t = weight of kid at time t,

a = intercept (initial body weight parameter),

b = regression coefficient (rate of gain),

t = time in week, and

e_t = random error term

2.3 Morphological Measurements

Body weights of goat turn as outcome of some fixed effects among them, management system, pregnancy, feed, season of birth, year, stage of lactation, age of dam, type of birth, parity, sex and breed effect. These factors are less affected to linear body confirmation. Furthermore, linear measuring is relatively simple to process in compare with animal weight as indirect method (Riva et al., 2004; Adeyinka & Mohammed, 2006)

2.3.1 Used Measurements

Height at withers (HAW)

Height of the withers is the distance from the peak point of the animal surface at the withers to the flat ground. It is better to use instrument made from two arms of wood, one of this arm is long vertical and other is short horizontal sliding strongly up and down on vertical centimeter scale index. The height at withers measure from shoulder at peak point by move shorter arm down until touches the shoulder.

Body length (BDL)

Length of the body is the distance from the origin of the ear to the origin of the tail. Body length can be measured also as the distance from origin of tail to beginning of the neck. Attention should be taken to ensure that the vertebral column is straight in both directions vertical and horizontal scale.

Hip width (HW)

Hip width is the distance between the outside edges of the hip bones on the right and left and measured with a pair of arm hinged and linked by a movable joint such as compass or caliper. One of these arms sliding on the centimeter index when released a way from the other giving distance between the two points.

2.4 Molecular Characterization

Seventy-four unrelated animals selected from accredited and commercial farms representing various regions of Saudi Arabia which raised Aradi breed were used in this study. Blood samples collected from the animals kept in Ice-tank using EDTA as anticoagulant. Then, DNA extraction began using ILLUSTRA blood mini spin kit (GE Life Sciences). After that, DNA samples were taking to determine the quantity and quality of DNA using Thermo NanoDrop 8000 UV-Vis Spectrophotometers. Twenty-nine selected microsatellites markers as shown in table 2 were used to study the molecular aspects in this way. Table 2 also showed characteristics for microsatellite markers reported for genetic variability analysis.

Markers were amplified using ProFlex™ PCR System Applied Biosystems. The PCR protocol are shown in table 3and PCR program shown in Figure 1.

Annealing temperature ranged from 55 up to 58 depending on primers sequence.

After PCR finished a vertical 8% polyacrylamide gel used in belong with 50bp DNA ladder to estimate allele sizes in base pairs (bp). A reference animal used to compare and correct allelic size in each gel.

2.5 Genetic Markers Polymorphism

To estimate the genetic variation, the following parameters were used: number of alleles, expected heterozygosity (He) and observed heterozygosity (Ho), calculated by Genetix 4.05 of Belkhir et al., 2004. Polymorphic information content (PIC) was calculated using program provide by Nagy et al. (2012).
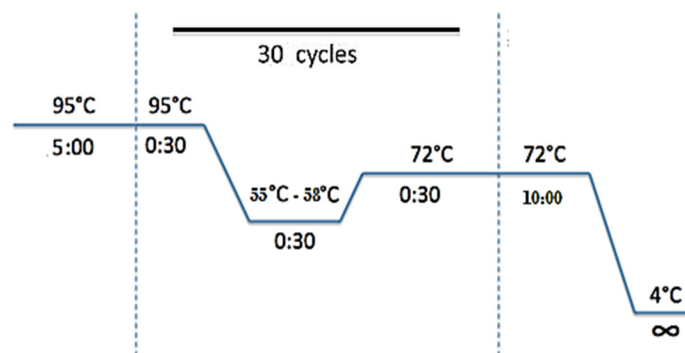

Figure 1. PCR program used to amplify each marker

Table 2. Characteristics* for 29 microsatellite markers reported for genetic variability analysis

Locus	H_0	H_e	Allelic Number	Size Range
INRA005	0.63	0.63	6	113-121
RM088	0.44	0.88	17	109-147
ILSTS002	0.70	0.82	12	113-135
CSRD247	0.69	0.76	11	220-248
INRA023	0.75	0.81	10	195-213
SRCRSP5	0.71	0.78	12	160-182
ILSTS011	0.75	0.77	9	264-280
MAF70	0.69	0.80	13	138-166
TGLA53	0.73	0.75	13	137-161
SRCRSP8	0.77	0.81	14	212-242
SRCRSP09	0.77	0.79	13	115-143
ILSTS044	0.35	0.52	8	142-177
OarAE129	0.83	0.74	9	130-175
ILSTS019	0.77	0.82	9	142-162
ILSTS033	0.49	0.75	12	151-187
ILSTS005	0.20	0.63	7	174-190
ILSTS058	0.71	0.94	23	136-188
ILSTS087	0.42	0.89	13	142-164
ILSTS034	0.25	0.39	6	153-185
ILSTS029	0.86	0.83	14	148-191
ILSTS049	0.44	0.71	9	160-184
ILSTS030	0.78	0.84	9	159-179
ILSTS082	0.98	0.84	15	100-136
ILSTS022	0.38	0.49	6	189-225
OARFCB48	0.80	0.82	11	149-181
SRCRSP1	0.85	0.70	13	113-143
OARCP34	0.86	0.71	13	102-132
BM2113	0.88	0.79	19	120-165
BM1818	0.77	0.79	10	253–272

*Data obtained from, Araújo et al. (2006); Dixit et al. (2010); Hoda et al. (2011); Kumar et al. (2009); Li et al. (2002); Seilsuth et al. (2016).

Table 3. PCR protocol

Components	volume
Genomic DNA (20ng),	2μl
PCR Master mix (applied Biosystems)	5μl
primer mix (50 pmoles)	0.4 μl
DNase free water	2.6 μl
Total Volume	10μl

3. Results and Discussion

3.1 Factors Effecting Weights

As shown in table 4, all fixed effects under study showed highly significant (P<0.01) in all 7 weights from birth to 24 weeks. These results were in agrement with that found by Khalil et al., 2010 and Mousa et al. (2013b).

3.2 Linear Model

The linear model was applied on male and female separately. The R^2 of that model was 0.996 and 0.993 in male and female, respectively. Table 4 show the Intercept *a* (initial body weight) and regression coefficient b (growth rate /per week) from regression analysis of weight in kg on time per (week) for male and female.

As shown in table 5, illustrate that the biological mean of intercept *a,* was the initial body weight (birth weight), were 3.84 Kg and 3.76 Kg for male and female, respectively. The biological mean of the b was growth rate, were 0.80 and 0.67 Kg /week for male and female, respectively. These results were close to (Mousa et al., 2013a) using parabola model on the same breed.

Figure 2 showed the observed values of the weight versus the predicted value from the linear equation. The predicted values of linear equation were slightly overestimated than observed in the initial body weight and weights from 20-24 weeks and slightly underestimate from 12-16 weeks in male and female. The correlation between true value of weights and predicted value were 0.998 and 0.996 male and female, respectively. The mean of square due to regression was highly significant (P<0.01) in male and female. To change the growth pattern to the best, run this equation for individually to get *a* and *b* for each animal and after that make a selection for the animal which have more than 3.84 kg as initial or more than 800 g/week as growth rate in week or both of them in male and by the same way in female.

Estimate of linear parameters in table 5 illustrate that the change in t (age of kid) by unite will be following by change in y (weight) by 800 g/week in male. This, mean that average daily growth rate was 114.28 g in male. Of course, the growth rate of kids is lower than lambs when comparing each other by the same age. But, the growth rate of Aradi goat is more than Sudanese Goat kids goat 87 g/day (Wilson, 1976) and East African goat 91g/day (Wilson, 1958). By the same way, the average growth rate in a week was 670 g (95.71 g/day) in female.

In General, the average growth rate for male and female kids were rapid or slow this according to the stage of age. The growth rate goes rapidly from birth to weaning weight showing straight line shape and start to decrease in growth rate as long as kids grows up in a lifetime showing curve linear shape (Mousa et al., 2010, 1989). The pattern of growth at this time it turns into a curved shape growth instead of linear shape.

The linear equation for predicting weights will be:

In male:

$$\hat{y} = 3.84 + 0.800t \tag{2}$$

In female:

$$\hat{y} = 3.76 + 0.670t \tag{3}$$

By substitute (t) at any time, will be obtain the predicted weight at this time.

Table 4. The significance effect of fixed factors year of season (Ys), litter size (LSB) and sex (sex) on all weights

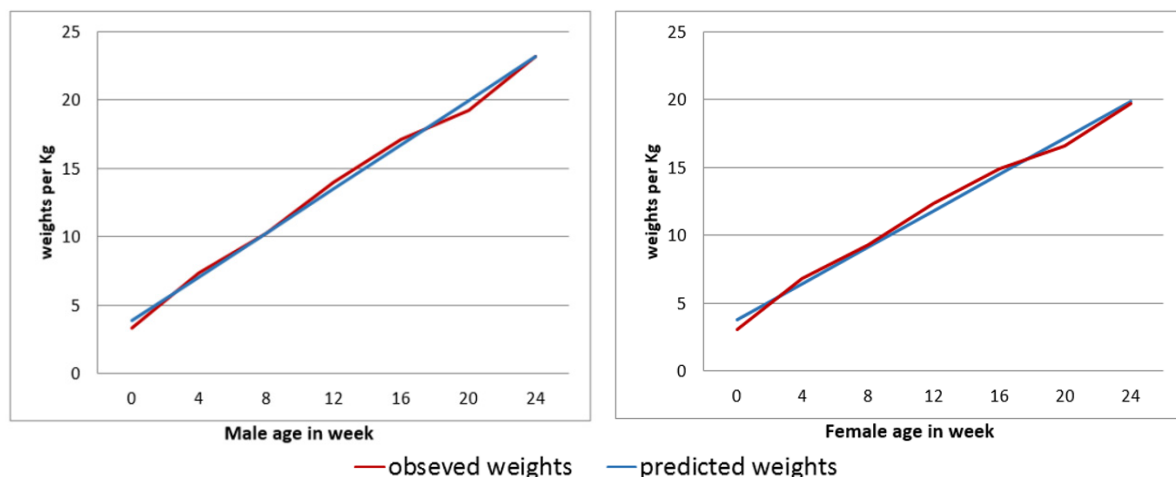|	Source of variation	Ys	LSB	Sex
	birth	2.35**	14.18**	16.78**
	4 week	1.95**	12.50**	7.63**
Weight at	8week	2.60**	26.28**	8.85**
	12 week	2.25**	17.05**	12.67**
	16 week	1.92**	7.58**	12.83**
	20 week	2.03**	5.64**	16.15**
	24 week	2.96**	4.12**	24.15**

** (p<0.01).

Figure 2. Observed weights versus the predicted weights by linear equation in Aradi kids.

3.3 Morphological Characterization

Aradi goats are dominate in northern of Saudi Arabia. The color is generally black or black with white spots around eyes nose and mouth. The face of Aradi Goat is Clean-cut with prominent bulging eyes and without beard. The body is long and barrel with straight and medium to short. The tail of Aradi is short curled upwards. The Aradi Goat has a medium-sized with integrated body. The coat of body is long rough hair and bristles. Horns are present in both sexes, which is semi-circular, bent backward in females, and may be helical in males, and long pendulous ears with Black dotted by white color.

Average body weights at 12 weeks were 13.96 and 12.34 Kg in male and female, respectively. Male and female kids had a least square mean 24-week weaning weight of 23.19 and 19.66 kg as shown in table 6 with the rest of body measurements according to Ediberto et al. (2011). As presented in Table 6, the height at withers for male (0.68) and female (0.62) was lower than reported by Bhattacharya (1989) because he did that measurement at older age (4-year) instead of 24 weeks in the present study. Also, the length of body is less than reported by Bhattacharya (1989).

Table 5. The Intercept *a* (initial body weight) and regression coefficient b (growth rate /per week) of regression analysis of weight on time per (week) for male and female.

Parameter	Male		Female	
	Value	P	Value	P
a	3.84±0.33	0.0001	3.76±0.36	0.0001
b	0.80±0.02	0.0000	0.67±0.03	0.0000

Table 6. Some phenotypic characterization of pure Aradi

Trait	Male	Female
Height (meter)	0.68±0.01	0.62±0.01
Length (meter)	0.86±0.02	0.80±0.01
Depth (meter)	0.34±0.01	0.30±0.01
Width (meter)	0.21±0.01	0.19±0.01
Birth weight (kilogram)	3.36±0.06	3.06±0.07
Weight at 12 week (kg)	13.96±0.38	12.34±0.47
Weight at weaning, 24 week (kg)	23.19±0.63	19.66±0.61

3.4 Molecular Characterization

Characterization of the 29 markers used in this study showed in Table 7, the maximum allelic numbers were found in loci CSRD247 and RM088 with 13 alleles. On the other hand, ILSTS022, ILSTS033, ILSTS030, ILSTS044, OARE129 loci showed the lowest alleles number 3 in each.

Observed heterozygosity, expected heterozygosity, represented in table 7. From the table, we can observe that observed heterozygosity values were lower than the expected one for ILSTS087, INRA023, TGLA53, SRCRSP8, CSRD247, RM088, ILSTS049, ILSTS002, BM1818, ILSTS034 and ILSTS033 loci, while it was higher than expected heterozygosity for the rest of markers. The average of observed heterozygosity was 0.63 ranged 0.89 in BM2113 locus to 0.17 in ILSTS002 locus. The heterozygosity level of each microsatellite marker varies from locus to another, the heterozygosity and number of alleles determine its polymorphic information content. The average PIC values (0.61) of the selected markers showed its benefits to study the genetic variability in goats.

As mentioned by Barker (1994) in the diversity studies the minimum allele number per each locus must be four in order to reducing the standard error for genetic distances, which reached in most used loci in the present study. Which were in agreement with Tekezaki and Nei (1996) which determined the range of usefulness markers to be used to estimate the genetic variation to be from 0.3 up to 0.8.

Also, this results are in agreement with that found by Dixit et al., (2012), in a similar study in 22 Indian goats, which found that the genetic diversity ranged from 0.61 in Osmanabadi up to 0.73 in Sirohi.

Table 6. Allelic number, Heterozygosity (observed and expected) and size range for studied markers

LOCUS	Allelic Number	H_0	H_e	PIC	Size Range
MAF70	12	0.76	0.83	0.73	129 - 163
ILSTS087	8	0.70	0.63	0.65	150 - 166
SRCRSP5	7	0.79	0.80	0.75	161 - 178
SRCRSP1	5	0.61	0.70	0.53	132 - 142
INRA023	7	0.80	0.61	0.77	190 - 206
TGLA53	8	0.61	0.57	0.58	129 - 147
OARFCB48	8	0.76	0.82	0.73	155 - 169
SRCRSP8	10	0.75	0.74	0.72	203 - 232
BM2113	10	0.81	0.89	0.79	125 - 150
CSRD247	13	0.85	0.63	0.84	225 - 253
ILSTS058	11	0.60	0.70	0.58	134 - 156
RM088	13	0.87	0.61	0.85	106 - 132
ILSTS005	5	0.61	0.72	0.54	190 - 198
ILSTS049	5	0.59	0.33	0.53	165 - 187
ILSTS002	7	0.52	0.17	0.49	117 - 135
ILSTS082	6	0.59	0.64	0.54	118 - 138
INRA005	6	0.76	0.82	0.72	110 - 122
SRCRSP9	7	0.72	0.73	0.68	120 - 140
BM1818	8	0.81	0.78	0.79	243 - 257
OARCP34	5	0.71	0.76	0.66	106 - 118
ILSTS029	11	0.72	0.74	0.68	147 - 175
ILSTS019	6	0.71	0.73	0.67	151 - 161
ILSTS034	7	0.57	0.52	0.52	164 - 184
ILSTS022	3	0.26	0.30	0.24	191 - 195
ILSTS033	3	0.49	0.46	0.44	160 - 164
ILSTS030	3	0.63	0.68	0.56	166 - 181
ILSTS044	3	0.66	0.72	0.58	150 - 166
ILSTS011	4	0.33	0.41	0.41	268 - 276
OARE129	3	0.25	0.28	0.22	151 - 155
Average	7.03	0.65	0.63	0.61	

The expected heterozygosity reached the maximum value in locus RM088 and the minimum one in locus OARE129, the average value was 0.65. The observed heterozygosity in this study was lower than the average (0.75) of the selected markers as presented in table 2. The difference between observed heterozygosity in this study and in reference may be due to the two loci OARE129 and ILSTS002 which showed low values of observed heterozygosity 0.28 and 0.17, respectively. By the other way, the average of expected heterozygosity as reported in table 2 was 0.66 are in agreement with that in the present study (0.65). Among the 29 studied loci, 24 markers showed high polymorphism, number of alleles ranged between four (ILSTS011) and 13 (CSRD247, RM088), while the other five loci had less than four alleles. As suggested by Barker (1994) each microsatellite markers used must have more than three alleles in order to reduce standard errors estimates.

The average of observed allelic number was 9.89 as reported by Iamartino et al. (2005) using 18 markers in Italian goat populations and 9.87 as reported by Glowatzki-Mullis et al. (2006) in 11 Switzerland goat breeds. By the other way, the number was reduced to 6.90 in Chinese goat breeds as reported by (Li et al., 2002) and 5.37 in Indian goat breeds as reported by Ganai and Yadav (2001).

Polymorphic information content (PIC) is a statistical measure to know the informativeness of a marker. Aggarwal et al. (2007), studied the Polymorphic information content in three populations Zalawadi, Gohilwadi and Surti the mean PIC values were 0.56, 0.64 and 0.60, respectively. Also, as reported by Botstein et al. (1980) Polymorphic information content values for any loci 0.5 or above is useful in the analysis of population genetic.

4. Conclusion

Primary investigation on function show that the linear equation was preferable one than the rest according to easy compute and have only two linear additive parameters which have biological interpretation. The accuracy (R^2) of that equation was very high ranged between 0.996 and 0.993 for predict weights in male and female, respectively.

Since, the predicted of future weights well be done accurately, the selection will be efficiency in kids. Aradi goats have a shape sets it apart from the rest of the other local breeds, which is the firmer dynasties in Saudi Arabia. Out of 29 microsatellites studied, the average of observed heterozygosity was 0.63 ranged from 0.89 in BM2113 locus to 0.17 in ILSTS002 locus. The expected heterozygosity reached the maximum value in locus RM088 and the minimum one in locus OARE129, the average value was 0.65. The polymorphic information content (PIC) values ranged from 0.85 (RM088) to 0.22 (OARE129). Thus, the investigation on a set at 29 microsatellites revealed high degree of genetic variability in Aradi goat indicating an important indigenous genetic resource.

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