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Relationship of Lactoferrin Gene with a Number of Growth Traits in Holstein Calves

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Abstract: ***This study was conducted at the Dairy Cattle Farm pertaining to the Department of Animal Production, College of Agriculture in Abu-Ghraib (20 km West of Baghdad) and Al-Jaderiah, as well as at the Biotechnology Physiology Laboratory at the College of Agriculture, University of Baghdad, Al-Jaderiah, in collaboration with the Laboratory specific for molecular genetic analysis during the period from 27/11/2015 to 1/ 04/2016. The objective of this study was to identify the polymorphism of lactoferrin gene and their relationship with some growth and productive traits for 28 Holstein cows and their births. The distribution percentage of lactoferrin gene polymorphism in cow's sample were 17.86 and 82.14 % for AA and AB respectively. The variation between these percentages were highly significant (P≤0.01). The allele frequency for A and B were 0.59 and 0.41 respectively, according to the lactoferrin gene analysis carried out currently. The effect of lactoferrin gene polymorphism.The body weight and dimensions of calves at birth did not affected by lactoferrin gene polymorphism. At weaning, the variation in heart girth was significant (P≤0.05), whereas, the weight and dimensions have not significantly influenced by lactoferrin gene polymorphism. The calf's body weight at 12 months old was 328.78 ± 5.99 kg for those born from AB cows, being greater (P≤0.05) than those born from AA cows (3.16.75 ± 10.19 kg). Moreover, AA genotype exhibited better (P≤0.05) service per conception, non-return rate and calving interval, along with interval between calving and subsequent successful mating (P≤0.01) than AB one. The calves mortality rate at weaning was significantly (P≤0.01) affected by lactoferrin gene polymorphism. It can be concluded from the study of gene expression for lactoferrin gene, the possibility of adopted them in cattle breeding strategy by increasing the economic gain of the breeding schemes, by selecting and crossing the high growth calves genotypes or phenotypes.***

Keywords: Lactoferrin gene; Holstein cows; Polymorphisms

1. Introduction

The milk is animportant food for the body due to contains the necessary nutrients that meetsourcesthe requirements of the consumer, who seek a high quality food products. It provides colostrum and milk for the new born's.the colostrum is a unique foodsource forinitial acquired immunity secreted withen 3 – 5 days immediately after birth because it has double amounts ofvitamins and minerals ratio as compared with the addition of milk inaddition of the diversity of milk protein, which includes immunoglobulin'sand intestines content of receptors have the ability to devour the antibodies and non- anole it to strengthen its immune. Both the immune concentration in colostrum and intestinal permeability are decrease rapidly during the first 24 hours after birth (Moore et al,2005, Butler,2005, Wheeler et al,2007). So it should accelerate to feed the new born's nascent quantities of antibodies through colostrum until the developing of the immune system self.

Milk protein is the best types of proteins found in nature due to it is easy to melt and rapid digestion and absorption as it contains all the amino acids and essential elements that cannot be produced by the bodies, it is important to know that the protein enters the building all the body tissues and that's where the protein components of casein, albumin and immunoglobulin.Lactoferrin isimmunoglobulin and it is important proteins, It is also important proteins and is part of the natural immune system, Which is distributed broadly through body fluids and secretions foreign as well as being the innate immune system(is the first Line of defense to protect the body from external causes enhanced protection against infections diseases and infections which result from granule cells neutrophil (White cells). Show high levels of lactoferrin in colostrum and milk (Masson and Hermans, 1971, Brock,1980). The mammary gland is controlling the amount of lactoferrin produced (Green and Pastewka, 1978). The variation in the level of Lactoferrin in milk through lactation stages leads to know where you have the highest level through the gene polymorphism and to create herds with high productivity level of the immune system and was born in the good health. A controversy still hostdefense mechanism and through the iron metabolism.

The genetic improvement processes have achieved significant results by studying the genotypes of these animals, through the study of genes affecting the production and knowledge of genetic mutations linked to phenotypic formulators using technology PCR (Polymerase chain reaction) and RFLP (Restriction Fragment Length Polymorphism), It helps in the study of genes required to determine the genotype of each animal and read the sequence of nitrogenous bases for animal DNA and detect the presence of mutations (Alain et al,2002,Lui and Cordess,2004), according to these techniques in the elections and in the state of education and the degree of genetic similarity within the strain and the study of the links between genetic multi-format and usability economic productivity genes (Rafay et al,2001). Accordingly, this study carried out to investigate a relationship between lactoferrin gene and some growth of Holstein new born.

2. Material and Methods

The study was conducted at the Dairy Cattle farm pertaining to the Department of Animal production, College of

Agriculture - Baghdad University, for detection of bovine Lactoferrin gene to molecular characterization of the gene to detect the gene phenotypic diversity and its relationship with some economic characteristics. The analysis is performed at the laboratories of Graduate Studies in the Faculty of Agriculture-Laboratory Technologies ecological vitality andphysiology, Baghdad University and laboratory Musayyib bridge in the Jadiriyah on blood samples for the duration of the 27/11/2015 and up 1/4/2016.The cows were reared in semi-closed pens .The nutrition was deferent with deferent season according to theavailability of roughages, alfalfa, yellow corn and clover in particular . The green roughages was introduce as available quantity and quality or dry roughage like hay and strew astwo meals, at morning and evening .The concentrate was also introduced as 2kg /cow, and increased to 3kg/cow during calving and lactation

Table 1: Chemicals used

X	Items	Manufacturer and origin
1	Agarose	Bio Basic
2	EDTA	AFCO(Jordan)
3	10Xtbe Buffer solution	Bio Basic
4	DNA marker	Bioneer (korea)
5	Bromophenol blue	Bioneer (korea)
6	primers	Promega (USA)
7	Extraction DNA kit	Promega (USA)
8	Polymerase chain reaction kit	Bioneer (korea)
9	Ethidium Bromide stain	Bio Basic
10	EcoR1 enzyme	Bioneer (korea)

3. Blood Samples

Blood samples for DNA extraction were collected via jugular venipuncture and placed in test tubes containing EDTA asananticoagulant and (EDTA) volume of 10 ml. the extracted DNA was stored at -20℃ until used.

Molecular analysis of lactoferrin gene:
The molecular analysis of lactoferrin gene was done in three stages ,1st stage was DNA extraction ,2nd stage wasextract the required piece from lactoferrin gene and amplify it, while the 3rd stage was cutting the required piece to determine the genotypes.

DNA extractions
Genome DNA was extracted from the whole blood.
This is isolated DNA was used for PCR amplification of the Lf gene fragment of 301bp with the use of the following primers,
Forward:5′-GCC TCA TGA CAA CTC CCA CAC-3′
Reverse:3′-CAG GTT GAC ACA TCG GTT GAC-5′

The gene name and abbreviation name	Primers	
Lactoferrin gene (Lf)	Exon 17	F: 5′-GCC TCA TGA CAA CTC CCA CAC-3′
		R: 3′-CAG GTT GAC ACA TCG GTT GAC-5′

Restriction analysis of the amplified fragment was done with RFLP using EcoR1 enzyme , the PCR produced three types of DNA fragments, with 301bp lengths of 100bp,201bp and 301bp.one DNA fragment with 301bp length was identified as an A allele, while that for two DNA fragment lengths of 201bp and 100bp was identified as a B allele.

4. Statistical Analysis

Analysis of polymorphic patterns in Lf gene was performed using the SAS-Statistical Analysis System(2012), to explore the effect of multiple genetic (genotypic fragment)of Lf gene of the recipes in milk production and component, were compared by the arithmetic means test (Duncan , 1955) multi-extent.

5. Results

Total DNA was extracted, at first step to isolate the target segment for Lf gene PCR-RFLP technique whereas samples were deported by 10µl blended with 2µl of loading dye within 1% agarose gel Adjust the voltage and current time and imaging out put relay to make ensure the success of the extraction process (Figure 1).

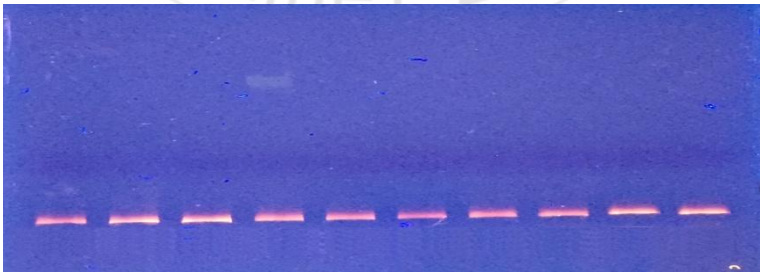

Figure 1: DNA extraction

PCR-RFIEP for genotype identification using animals genotype was using restrictionfragment length Polymorphism (RFLP) and EcoR1 restriction enzymes, then electrophoresed on 2.5% agarose gel in a voltage of 70 volts for half and one hours. After electrophoresis was completed the agarose gel was taken to see the length of the DNA bands using ultraviolet light then compared with the marker (Figure 2).

Genotype AA It appears in the columns 3,4,10,11
Genotype AB It appears in the columns 1,2,5,6,7,8

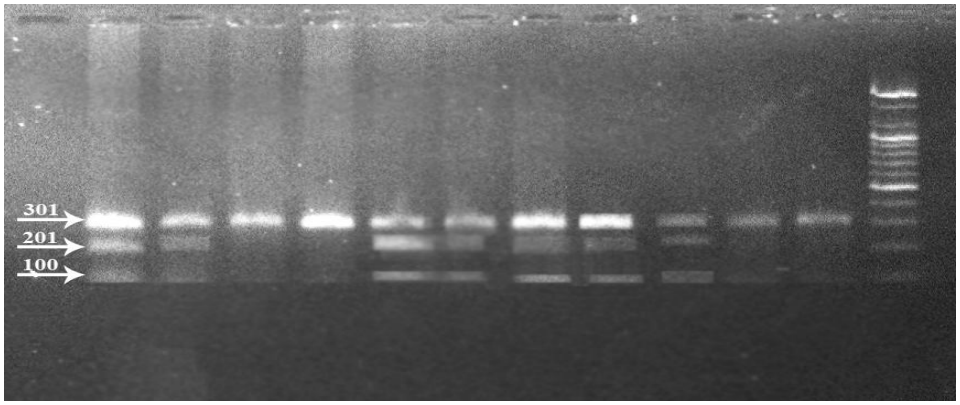

Figure 2: Fragment after restriction EcoR1 enzyme to determination Lactoferrin gene

Effect of lactoferrin gene polymorphism for cows on the bodyweight and dimensions of new born's calves

Results revealed that LFgene polymorphism did not has any significant effect on body at birth ,namely 32.20±1.06 and 32.15±1.17 for AA and AB respectively ,Moreover LF genes polymorphism did not alter body length and heart girth ,A significant (P≤0.05) difference was noticed for height at front ,being 75.69±0.89cm for AB and 71.60± 0.89cm for AA (table 2) . ,

Table 2: Effect of lactoferrin gene polymorphism on the body weight and dimensions of new born calves(Means ± SE).

	Lactoferrin genotype		
Trait	AA	AB	Level of sign.
Body weight at birth(kg)	32.20 ±1.06a	32.15 ±1.17a	NS
Body length (cm)	54.60 ±1.50a	54.34 ± 0.65a	NS
Heart girth(cm)	67.00 ± 2.79a	68.47 ±1.18a	NS
Height of front (cm)	71.60 ±1.12a	75.69 ± 0.89a	*
Average that carry different letters within the same column significantly differ among themselves. *: (P<0.05), NS: non significance			

Effect of lactoferrin gene polymorphism for dams on the new born body weights and dimensions at weaning

the current results of LF gene polymorphism revealed that body weight and length and height of at front did not affected with different with different polymorphism , variation was significant (P≤0.05) for heart girth ,being 107.08±0.89 cm in AB and 101.60±2.11for AA (table 3).

Table 3: Effect of lactoferrin gene polymorphismonthe new bornebody weights and dimensions at weaning (Means ± SE)

	Lactoferrin genotype		
Trait	AA	AB	Level of sign.
Body Weight at weaning (kg)	101.60 ± 2.15a	101.26± 2.21a	NS
Body length (cm)	76.80 ± 1.15a	77.69 ± 0.61a	NS
Heart girth (cm)	101.60 ± 2.16b	107.08± 0.89a	*
Height at front (cm	99.80 ± 2.06a	99.00 ±1.08a	NS
Average that carry different letters within the same column significantly differ among themselves. *: (P<0.05), NS: non significance			

Effect of lactoferrin gene polymorphism on average body weights at 6,9 and 12 months of age

Non significant different were observed for 6 and 9 month of age on average body weight , while it appear at 12 month of age being 328.78±78 for AB and 316.75±10.19 for AA.(Table 4)

Table 4: Effect of lactoferrin gene polymorphismon average body weights at the 6,9 and 12 months of age (Means ± SE)

	Lactoferrin genotype		
Trait	AA	AB	Level of sign.
Weightat the age of 6months (kg)	166.20 ± 4.97a	167.04 ±3.65a	NS
Weight at the age of 9months (kg)	242.40± 8.52a	243.80 ±5.97a	NS
Weight at the age of 12months (kg)	316.75±10.19b	328.78 ±5.99a	*
Average that carry different letters within the same column significantly differ among themselves. * :(P<0.05), NS: non significance			

Table 5: Effect of lactoferrin gene polymorphism on weight gain between the different ages of calves (Means ± SE)

	Lactoferrin genotype		
Trait	AA	AB	Level of sign.
Weight gain from birth to weaning(kg)	69.60±4.56	69.11±1.81	NS
Weight gain from birth to 6 months(kg)	134.00±4.32	134.89±3.37	NS
Weight gain from weaning to 6 months (kg)	64.40±1.28	65.78±3.08	NS
Weight gain from birth to 9 months (kg)	210.20±7.57	210±5.56	NS
Weight gain from weaning to 9 months(kg)	140.60 ±6.86	141.82±5.13	NS
Weight gain from 6 to 9 months(kg)	76.20±6.01	76.04±2.77	NS
Weight gain from birth to 12 months(kg)	281.60±9.23	284.63±10.55	NS
Weight gain from weaning to 9 months(kg)	212.00±8.90	215.52±10.28	NS
Weight gain from 6 to 12 months(kg)	147.60±7.95	149.74±8.38	NS
Weight gain from 9 to 12 months(kg)	71.40±2.18	73.69±6.51	NS
NS: non significance			

Effect of lactoferrin gene polymorphismon calve mortality percentage till the weaning

Table (6) showed highly significant (P≤0.01) effected of LF gene polymorphism on calves mortality percentage being 20.57 2.75 for AA and 8.70 0.72 for AB.

Table 6: Effect of lactoferrin gene polymorphismoncalves mortality percentage from births to weaning

The percentage of mortality from births to age weaning(means ± S.E)	Number	genotype
20.57± 2.25a	5	AA
8.70 ± 0.72b	23	AB
**	--------	Level of sign.
Average that carry different letters within the same column significantly differ among themselves. ** (P<0.01)		

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