β-Lactoglobulin, Kappa Casein, and Prolactin Genes Effects on Milk Production

Khaleel I. Z. Jawasreh & Ahmad H. Almareen

Jordan University of Science and Technology, Jordan and National Agricultural Research Center

Introduction

- The dairy industry is a crucial cog in the agricultural economy of many countries.
- Therefore, milk yield and its components are among the major goals targeted by animal geneticists.
- Awassi is the most common breed of sheep in Middle East countries where its products,

Introduction

- **Beta-lactoglobulin,** coded by the *β-LG* gene, is synthesized by the secreting cells of the mammary gland.
- *β-LG* is the primary whey protein in ruminant's milk and accounts for approximately 17 to 22% of total milk proteins.
- The *β-LG* gene is located on ovine chromosome 3, and exon number 2 of *β-LG* revealed three allelic polymorphisms (A, B and C) based on different amino acid changes.
- The alleles A and B (Tyr/His) differ at the amino acid position and the genetic variant C differs from variant A by an amino acid exchange at position 148 (Arg/Gln) (GenBank accession No. X12817).
- The most common genetic variants detected in all studied sheep breeds are A and B, while the variant C is regarded as rare,

Introd.

- Prolactin, coded by the *PRL* gene, is a lactogenic hormone found in many species.
- The *PRL* gene plays a key role in the development of the mammary gland and milk secretion; its depletion in sheep provokes a severe reduction in milk production , suggesting that *PRL* is a functional candidate gene contributing to variations in milk production.
- The *PRL* gene is found in a region of the ovine chromosome 20 with putative quantitative trait locus (QTL) for milk yield and composition.
- Thus, PRL is primarily responsible for the synthesis of fat, proteins, and all other major components of milk .

Introd.

- The four caseins (*αS1-, αS2-, β- and κ-casein*) are the major proteins in sheep milk, accounting for about 80% of total protein in milk.
- Among the 4 caseins, *κ-casein* (*CSN3*) accounts for approximately 15% of total casein,
- and thus represents one of the most important proteins due to its essential role in micelle formation and stabilization,
- determines the manufacturing properties of milk

Objectives

- The present study screens some Awassi genetic loci for possible variants in the *β-LG, PRL*, and *CSN3* genes
- and establishes their frequency in commercial Awassi sheep flocks
- The ultimate objective **of investigating the effect of these genotypes and their interaction** on milk production and composition traits.

Materials and Methods

- Animal and Sample Collection

✓ A participatory animal-breeding program was performed in the South, North, and middle of Jordan.

✓ .A total number of 928 ewes that belong to 9 flocks (three in each region) were targeted through the participatory animal-breeding program.

- Blood samples for DNA harvesting were carefully collected from the jugular vein of the Awassi ewes (2 to 6 years old) that were born to 31 sires using vacuum tubes and stored at −20.

Milk Samples and Analysis

- Milk was collected manually (hand milking) by skilled workers.
- Milk samples were taken also biweekly for milk component analysis

Results

Genomic DNA Extraction and Polymerase Chain Reaction (PCR)

Table Primer information and restriction information for genes of interest.

Gene		Primers (5′→3′)	TM (°C)	PCR Product (bp)	RE
Beta-lactoglobulin (*β-LG*)	F	CTCTTTGGGTTCAGTGTGAGTCTTG	58	301	RsaI
	R	CACCATTTCTGCAGCAGGATCTC			
Prolactin (*PRL*)	F	ACCTCTCCTCGGAAATGTTCA	56	1209	HaeIII
	R	GGGACACTGAAGGACCAGAA			
Kappa Casein (*CSN3*)	F	CTGGGTTCACTATTCCCAATG	57	680	Sequencing
	R	TTGCTCATTTACCTGCGTTG			

Table Descriptive statistics of milk production and milk composition traits.

Descriptive statistics of milk production and milk composition traits.

Milk Trait	**No. Records**	**Mean**	**SE**	**CV (%)**
Milk production (Kg)				
TMY	391	96.8	2.60	53.0
TDM	391	0.882	0.02	45.0
Milk composition				
Fat%	917	5.80	0.05	25.7
SNF%	986	9.74	0.03	8.30
Protein%	986	3.90	0.02	13.0
Lactose%	986	5.10	0.02	13.9
Density g/cm^2	986	34.3	0.10	9.4

Genotyping

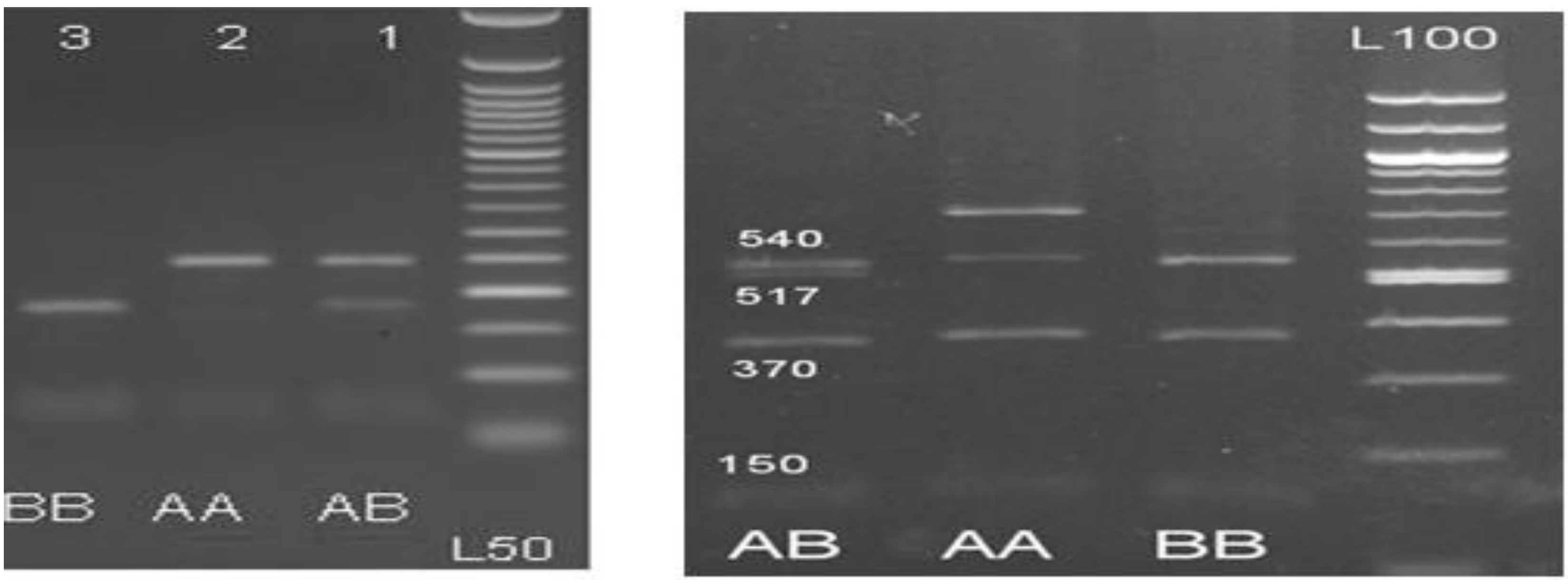
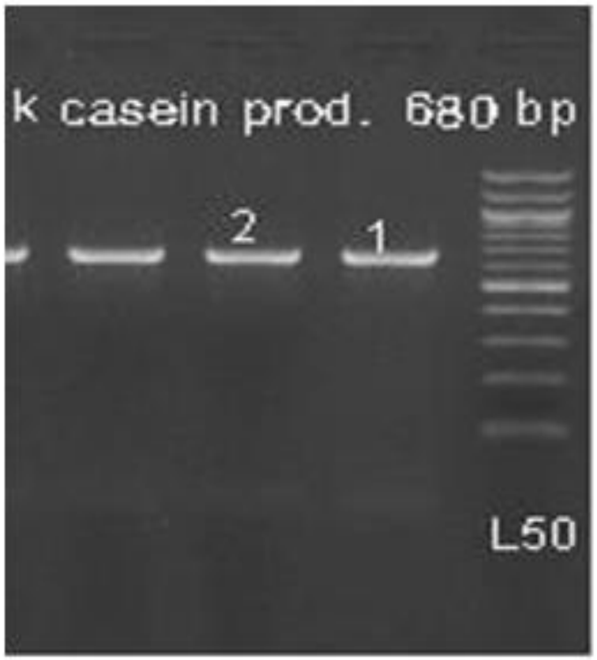
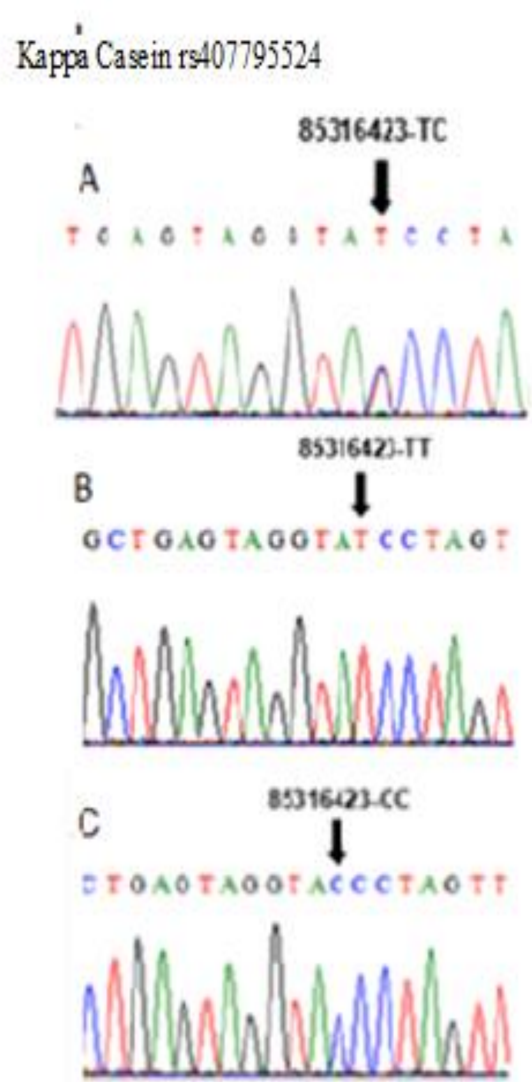
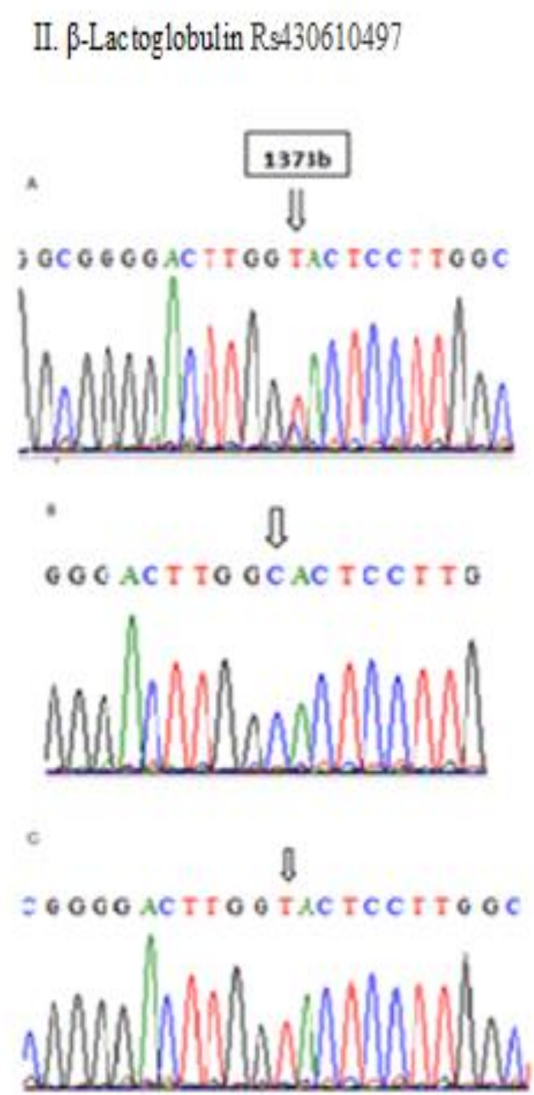


Figure 1. PCR-RFLP results for *β-LG* (**A**) and prolactin (**B**) genes using RsaI and HaeIII restriction enzyme respectively on 3% agarose gel

sequencing results







Allele and genotype Frequencies

Gene [1]	Genotype	Observed Number	Expected Number	Genotype Frequency	Allele	Allele Frequency	Value of x^2 Test
***β-LG* (n = 159)**	**AA**	27	28.7	0.17	A	0.42	0.29 ns
	AB	81	77.7	0.51	B	0.58	
	BB	51	52.7	0.32			
***PRL* (n = 158)**	**AA**	115	107	0.73	A	0.82	19.2 ns
	AB	30	46.1	0.19	B	0.18	
	BB	13	5	0.08			
***CSN3* (n =156)**	**TT**	132	132.9	0.85	T	0.92	1.1 ns
	TC	24	22.1	0.15	C	0.08	

P-Values

Trait / Factors	Milk Production [1]		Milk Components [2]				
	TMY (kg)	**TDM (kg)**	**Fat%**	**SNF%**	**Protein%**	**Lactose%**	**Density, g\cm^2**
β-LG	0.175	0.134	0.047	<0.0001	0.046	0.03	0.002
PRL	0.034	0.011	0.761	0.001	0.035	0.05	0.005
CSN3	0.812	0.275	0.172	0.048	0.424	0.104	0.541
Sire	<.0001	<.0001	<.0001	<0.0001	0.019	0.035	0.0004
Parity	0.004	0.005	0.056	0.412	0.389	0.266	0.665
Year	0.003	0.002					
β-LG×PRL	0.039	0.031	0.001	<0.0001	0.008	0.035	0.05
β-LG×CSN3	0.874	0.221	0.417	0.899	0.784	0.949	0.496
PRL×CSN3	0.177	0.104	0.002	0.02	0.228	0.115	0.767
Dam weight at lambing	0.299	0.009					

Table Effect of Beta lactoglobuline (*β-LG)*, Prlactin (*PRL)*, and *Kappa casein (CSN3)* genotypes and significant interaction effects on milk production traits in Awassi sheep.

Gene	Genotype	N	Trait Least Square Means (± SE)	
			TMY (Kg)	TDM (Kg)
β-LG	AA	51	100.4 ± 13.4	0.718 ± 0.10
	AB	145	72.2 ±15.7	0.606 ± 0.10
	BB	96	93.2 ± 12.6	0.801 ± 0.10
PRL	AA	197	102.4 ± 9.86[a]	0.814 ± 0.08 [a]
	AB	68	71.4 ± 12.6 [b]	0.540 ± 0.10 [b]
	BB	27	92.0 ± 14.7 [ab]	0.770 ± 0.11 [a]
CSN3	TT	240	90.1 ± 11.8	0.762 ± 0.09
	TC	52	87.1 ± 11.5	0.654 ± 0.09
β-LG×PRL	AAAA	29	96.5 ± 13.0 [b]	0.780 ± 0.10 [ab]
	AAAB	12	65.0 ± 23.0 [bc]	0.359 ± 0.17 [c]
	AABB	10	139.7 ± 25.2[a]	1.02 ± 0.19 [a]
	ABAA	106	99.2 ± 17.3 [ab]	0.834 ± 0.13 [ab]
	ABAB	31	72.6 ± 19.3 [bc]	0.578 ± 0.15 [c]
	ABBB	8	44.9 ± 18.4 [c]	0.406 ± 0.14 [c]
	BBAA	62	111.6 ± 11.1 [ab]	0.829 ± 0.08 [ab]
	BBAB	25	76.8 ± 15.4 [bc]	0.684 ± 0.12 [b]
	BBBB	9	91.3 ± 27.6 [ab]	0.891 ± 0.21 [ab]

Least Square Means of Milk components and the genes effects

Gene	Genotype	N	Traits Least Square Means (± SE)				
			Fat %	SNF %	Protein %	Lactose %	Density, g\cm^2
β-LG	AA	112	6.63 ± 0.29 ^{a}	9.50 ± 0.15 ^{b}	3.90 ± 0.10 ^{b}	4.99 ± 0.14 ^{b}	33.0 ± 0.61 ^{b}
	AB	376	5.31 ± 0.43 ^{b}	9.39 ± 0.22 ^{b}	3.66 ± 0.14 ^{b}	4.88 ± 0.20 ^{b}	34.1 ± 0.87 ^{ab}
	BB	277	6.30 ± 0.35 ^{a}	10.4 ± 0.18 ^{a}	4.13 ± 0.12 ^{a}	5.39 ± 0.16 ^{a}	35.4 ± 0.71 ^{a}
PRL	AA	554	6.13 ± 0.17	9.86 ± 0.08 ^{a}	4.00 ± 0.06 ^{a}	5.02 ± 0.08 ^{b}	34.2 ± 0.34 ^{a}
	AB	159	5.96 ± 0.19	9.44 ± 0.10 ^{b}	3.79 ± 0.06 ^{b}	4.90 ± 0.09 ^{b}	32.9 ± 0.40 ^{b}
	BB	52	6.15 ± 0.38	9.96 ± 0.19 ^{a}	3.91 ± 0.13 ^{ab}	5.35 ± 0.18 ^{a}	35.3 ± 0.77 ^{a}
CSN3	TT	624	6.49 ± 1.45	10.1 ± 0.19 ^{a}	3.98 ± 0.12	5.32 ± 0.17	34.5 ± 0.75
	TC	141	5.67 ± 1.05	9.45 ± 0.15 ^{b}	3.82 ± 0.10	4.86 ± 0.14	33.7 ± 0.62

Gene	Genotype	N	Traits Least Square Means (± SE)				
			Fat %	SNF %	Protein %	Lactose %	Density, g\cm²
β-LGxPRL	AAAA	56	6.38 ± 0.34 bc	9.77 ± 0.17 b	4.05 ± 0.11 b	4.87 ± 0.16 c	33.6 ± 0.70 b
	AAAB	29	5.56 ± 0.45 c	8.63 ± 0.21 c	3.47 ± 0.14 c	4.46 ± 0.19 d	30.2 ± 0.84 c
	AABB	27	7.95 ± 0.82 a	10.1 ± 0.42 b	4.18 ± 0.28 ab	5.66 ± 0.39 ab	35.2 ± 1.71 ab
	ABAA	284	5.97 ± 0.24 c	9.87 ± 0.11 b	3.99 ± 0.08 b	5.06 ± 0.10 bc	34.6 ± 0.46 b
	ABAB	78	6.67 ± 0.30 b	9.97 ± 0.15 b	3.97 ± 0.10 b	5.24 ± 0.14 b	34.5 ± 0.61 b
	ABBB	14	3.28 ± 1.11 d	8.33 ± 0.57 c	3.02 ± 0.38 c	4.35 ± 0.50 cd	33.3 ± 2.30 bc
	BBAA	214	6.03 ± 0.18 c	9.92 ± 0.09 b	3.96 ± 0.06 b	5.12 ± 0.08 bc	34.6 ± 0.36 b
	BBAB	52	5.63 ± 0.38 c	9.72 ± 0.15 b	3.91 ± 0.10 b	4.99 ± 0.14 bc	34.1 ± 0.59 b
	BBBB	11	7.23 ± 1.03 ab	11.5 ± 0.52 a	4.53 ± 0.35 a	6.05 ± 0.48 a	37.4 ± 2.10 a
PRLxCSN3	AATT	478	6.13 ± 0.18 b	9.89 ± 0.08 b	3.95 ± 0.05	5.11 ± 0.07	34.5 ± 0.32
	AATC	75	6.12 ± 0.27 b	9.82 ± 0.13 bc	4.05 ± 0.09	4.93 ± 0.12	33.9 ± 0.54
	ABTT	122	5.32 ± 0.22 c	9.29 ± 0.11 d	3.68 ± 0.46	4.83 ± 0.17	32.9 ± 1.02
	ABTC	37	6.59 ± 0.32 b	9.59 ± 0.16 c	3.89 ± 0.11	4.68 ± 0.15	32.9 ± 1.45
	BBTT	23	8.02 ± 1.11 a	11.0 ± 0.57 a	4.31 ± 0.38	6.03 ± 1.12	36.2 ± 2.30
	BBTC	29	4.29 ± 0.75 d	8.93 ± 0.38 d	3.51 ± 0.62	4.68 ± 0.35	34.5 ± 1.55

Conclusion

- The findings presented in this paper indicated that *β-LG* gene polymorphisms are not associated with milk production traits, but rather with varying fat%, protein%, SNF%, lactose%, and even density.
- PRL gene polymorphism was associated positively with SNF%, lactose% and milk density but not fat% or protein%. Furthermore, there was no *CSN3* variants effects on milk production or composition traits in Awassi ewes.
- **The interesting portion of this project** was the combined genotype effect on milk production and composition
- where we showed a significant impact of the interaction of *β-LG*^{x}*PRL* genotypes on milk production and of PRLx*CSN3* on fat% and SNF%,

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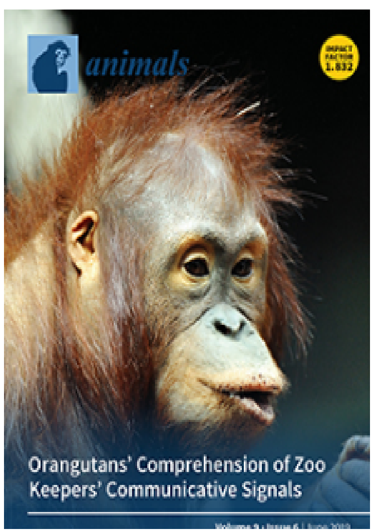

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Effect and Interaction of β-Lactoglobulin, Kappa Casein, and Prolactin Genes on Milk Production and Composition of Awassi Sheep

Khaleel Jawasreh [1,*], Ahmad Al Amareen [2] and Pauline Aad [3]

[1] Department of Animal Production, Jordan University of Science and Technology (JUST), Irbid 22110, Jordan
[2] Livestock Directorate, National Agriculture Research center (NARC), Albaqa'a 19381, Jordan
[3] Department of Sciences, Notre Dame University Louaize, Zouk Mosbeh 1211, Lebanon
* Author to whom correspondence should be addressed.

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