Aims and Scope

Our Institute Lalahan International Center for Livestock, Research and Training has been operating in the field of Animal Science and Livestock since 1951. Among the livestock activities, our Institute continues its activities in the fields of cattle breeding, ovine breeding and poultry breeding. In addition Institute's Breeding, Animal Nutrition, Genetics, Artificial Insemination and Embryo laboratories actively serve. Numerous research projects have been completed or still continue to be carried out in these areas. Institute has a journal named "Lalahan Livestock Research Institute Journal" which has been publishing 2 issues per year since 1959. The journal has the status of a National Refereed Journal followed by ULAKBİM (Turkish Academic Network and Information Center) in the field of Livestock. The journal, which has a strong archive and knowledge in its field, will continue its publication in English in order to carry it to International Standards. The journal will continue its publishing life as its new name 'Livestock Studies'.

LivestockStudies covers all kind of studies related to farm animals from poultry and bees to cattle, sheep, goats, etc. as follows:

Livestock Studies has been monitored: ULAKBIM (Turkish National Academic Network and Information Center), FAO AGRIS, CAP Abstract, CABI Full Text, Animal Breeding Abstracts, Google Scholar.

Further information for "**Livestock Studies**" will be published biannually in July and December for the above mentioned subjects.

LivestockStudies is a fully open access journal, with free online access to all content. No page charges or publication fees are collected. There is no payment at any stage of the article publishing process.

Publishing Date: December 2020

Pressed by MEDİSAN Publication Co.
Phone: +9 0312 311 00 57 -0536 577 22 44 - medisanyayinevi@gmail.com

---

Corresponding Address

Address : International Center for Livestock Research and Training (Uluslararası Hayvancılık Araştırma ve Eğitim Merkezi Müdürlüğü)
Lalahan Mah. S. Sırrı İçöz Cad. Mamak - Ankara / Turkey Web : http://arastirma.tarimorman.gov.tr/lalahanhmae

E-mail : info@livestockstudies.org

Phone : +90 312 865 11 96

Fax : +90 312 865 11 12

LIVESTOCK STUDIES

Editorial Information

LIVESTOCK STUDIES
Volume 60 Issue 2 December 2020

CONTENTS

Livestock Studies, 2020; 60 (2): 37-45
http://doi.org/10.46897/livestockstudies. 846383

RESEARCH PAPER

LIVESTOCK STUDIES

The effects of adding inactive yeast culture (*Saccharomyces Cerevisiae*) to rations prepared with different quality roughages on fattening performance, nutrient digestibility, some rumen parameters and carcass yield in lambs

Duygu Budak[1*], Aydan Yılmaz[2]

[1] Aksaray University, Faculty of Veterinary Medicine, Department of Zootechnical and Animal Nutrition, Aksaray, Turkey.
[2] Ankara University, Faculty of Agriculture, Department of Feeds and Animal Nutrition, Ankara, Turkey.

Article History
Received: 16 June 2020
Accepted: 18 November 2020
First Online: 25 December 2020

Corresponding Author
Tel.: +903822882862
E-mail: budakduygu@aksaray.edu.tr

Keywords
Roughage
Inactivated yeast
Digestibility
Rumen parameters
Temperature
Lamb

Abstract

This study was performed with the participation of 26.22±1.07 kg average weighted 36 singleton lambs. 6 Groups were formed; 3 with IYM (7 g/day) and 3 without IYM. Lambs were kept in individual divisions and given 150 g/day roughage (alfa alfa hay (AH), meadow hay (MH), wheat straw (WS), concentrated feed and water ad libitum. Treatment lasted 70 days in total; temperature and humidity were recorded daily. Fattening performance, carcass parameters and ruminal pH levels were not affected by the treatment (P>0.05). Additive increased the weight of the hearts which were fed with AH (P<0.05). Effects of roughage and additive interactions on the acetic, propionic and volatile fatty acids was found as significant (P<0.01). IYM, increased the rates of acetic acid/propionic acid feeding with AH and MH (P<0.05). Ammonia concentration and digestibility of crude cellulose and hemicellulose was affected by the type of roughage (P<0.05). Digestibility of acid detergent fibre (P<0.01) and cellulose (P<0.001) was increased with additives. There is no difference among the groups in terms of water consumption (P>0.05). It's concluded that, temperature and humidity is an efficient environmental factor on the fattening performance and IYM have positive effects on some cell-wall components digestibility and rumen parameters.

Introduction

Roughage is a stimulant factor in all processes of the digestion system of developing ruminant from the development of microflora to obtaining efficiency. However, as this stimulant is not sufficient it is emphasized that age and genus of the animal and usage of expedient feed additives together can create important effects. IYM are distinct among feed additives for their natural and biotechnological feature. Inactive yeast are probiotic metabolites as a good vitamin and mineral source produced mostly as high biologic value proteins after the fermentation of probiotic live yeast cells in an anaerobe culture medium with carbohydrate (Inge *et al*. 2009, Ezema 2013, Anonymous 2014a,b). By means of improving the adaptation ability of farm animals to poor conditions and increasing their resistance against diseases and their genetic potentials they serve as potential alternatives for antibiotics. (Abd-El Ghani 2004, Moharrery and Asadi 2009). In recent years it has drawn attention for its decreasing effects on metabolic disease risks (Vyas *et al.* 2014).

It is reported that by using IYM in ruminant rations more benefit can be provided than roughage especially of low-quality (Tripathi and Karim 2011, Zain *et al.* 2011). It is emphasized those effects result from the modification of rumen fermentation and thus they depend

on the composition of ration used (Kocaoglu and Kara 2010, Anonymous 2014a, b). Therefore, enhancement is achieved in the rate of animal's utilization from feed (Karademir and Karademir 2003, Turkmen *et al.* 2011) and it is observed that digestibility of some nutrients are increased (Haddad and Goussous 2005, Ghoneem and Mahmoud 2014). Effects of IYM on rumen parameters were associated with the quality of roughage aside from ration composition primarily (Opsi *et al.* 2012, Jurkovich *et al.* 2014). Also, its effects are expected on preserving the pH level of rumen by consuming the oxygen produced by aerobic pathogen (Ghoneem and Mahmoud 2014) and transforming ammonia nitrogen into microbial protein by increasing the density of bacteria in rumen (Patra 2012).

On the other hand, it is emphasized that ambient temperature and humidity is an important environmental factor where ruminants can easily maintain their body temperature during growing and development periods (Niyas *et al.* 2015) and the effects of ration composition against variable environment factors are a question of interest.

Through this study it is aimed to determine the effects of supplementation of inactivated yeast metabolites (IYM) (*Saccharomyces cerevisiae*) to rations containing alfalfa hay (AH) meadow hay (MH) and wheat straw (WS) for Anatolian Merino Sheep in terms of fattening performance, digestibility of nutrients, some rumen parameters and carcass yield.

Materials and Methods

This study was carried out with the decision of the local ethics committee of animal experiments in ankara university with the code 2014-22-151.

Trial Plan and Feeds

Animal materials; comprised of 26.22±1.07 kg average live weight and weaned at age of 2,5 months 36 singleton male Anatolia Merino lambs. In the study which was performed between September and November (out of weaning season) lambs were weighed at the beginning of trial and grouped according to their live weights homogeneously and randomly. For each roughage used in rations 6 trial groups were formed; 3 with IYM and 3 without IYM. During the study performed on factorial experiment design basis (3 roughages x 2 levels) lambs were fed in individual partitions for 70 days; 10 days being the adaptation period to the feed and the following 60 days for main fattening period. Lambs were given 150 gr 3-5 cm size chopped roughage (by considering the feeding of dry matter equal to roughage) (NRC 2007) and IYM was added to the roughage for 7 g/day of the groups taking additive feed (Anonymous 2015). After the mixture of yeast metabolite and roughage was consumed by the animals, concentrate feed and clean water were provided *ad-libitum.* Lamb fattening feed (LFF) was supplied from a feed factory. Inactive Yeast Culture was used as inactivated yeast metabolite (IYM) (Anonymous 2015).

A.O.A.C. 1984 was used as the method of determining the values of dry matter (DM) crude protein (CP), crude cellulose (CC), ether extract (EE) and crude ash (CA); Van Soest, 1994 procedure was followed for determining the amounts neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL); and for IYM Anonymous 2015 values were used. Metabolic energy (ME) (Anonymous 2016), organic matter (OM=DM-CA), non-nitrogen extract (NNE=DM-(CP+CA+EE+CC)), hemicellulose (HCL=NDF-ADF), cellulose (CL=ADF-ADL) and lignin (L= ADL-insoluble ash) values were derived from the analysis results on feed materials through calculation (Table 1).

Table 1. Chemical composition of feed and inactivated yeast metabolites used in the study

	AH	MH	WS	LFF	IYM
ME,Mcal/kgDM	1.38	1.76	1.37	2.73	-
DM, %	93.25	93.35	92.90	90.44	-
OM, %	85.09	81.39	83.5	82.22	-
CP, %	11.55	9.16	4.20	17.41	15.0
CC, %	38.98	27.56	37.37	9.73	-
EE, %	0.99	1.60	1.09	2.85	3.0
CA, %	8.16	11.96	9.40	8.22	6.0
NNE, %	33.57	43.07	40.84	52.23	-
NDF, %	62.73	60.70	75.02	41.43	-
ADF, %	34.00	44.52	53.69	12.61	-
ADL, %	9.76	10.04	12.36	6.38	-
HCL, %	28.74	16.17	21.33	28.82	-
CL, %	24.24	34.48	41.33	6.23	8.0
L, %	8.46	7.78	10.09	5.14	-

Temperature and Humidity

During the trial outdoor and in-barn temperatures (°C) and percentage of in-barn humidity (%) were measured every day at exact times (07:00, 14:00 and 21:00), thus the maximum and minimum temperatures were determined together with the in-barn humidity values. In consideration of those values and differences of maximum-minimum temperatures and humidity; daily average temperature and humidity values were calculated. Daily average temperatures were calculated based on the formula of Dağsöz and Bayraktar 1999 and humidity values were derived from the average of three measurements.

Daily average temperature (°C) = (t7 + t14 + 2 x t21) /4 t (hour): temperature measured at 7, 14 and 21

Parameters of Fattening Performance

Live weight (LW) values were measured on individual basis at the beginning of the feeding period and were continued to be recorded every two weeks until the end of the trial. Animals were weighted before feeding in the mornings at the same time (08:00). 12 hours before the weighing lambs were left hungry. Live weight gain (LWG) were measured daily and separately in all trial periods during the study. Individual feed consumption of lambs was calculated daily by taking the difference between the weight of morning feed and the amount left after feeding. Daily live weight gain was calculated by using these recorded data. Based on the data collected from this procedure, feed convertion ratio (FCR) was calculated.

Digestibility Parameters and Water Consumption

Manure collecting method was used for the determination of in vivo digestibility for DM, OM, CP, CC, EE, CA, NNE, NDF, ADF, HCL and CL rations (Akyildiz 1984). For this purpose, during the last 10 days of fattening every morning before feeding lamb's stool collected from the zippered manure bags which were attached to them. 10% of collected stool was weighted wet and the remaining part was weighted after drying in drier chamber at 60°C for 72 hours and then grinded as to pass a 2 mm sifter. Samples were taken for ten days from each lamb and 360 samples were obtained in total. After those 10-day samples were combined for each lamb 36 samples were analysed. All the manure samples ready for analysis were studied with (A.O.A.C. 1984) method for DM, CP, CC, EE and CA analysis and (Van Soest 1994) for NDF, ADF and ADL analysis. Based on the results of these analysis OM (OM=DM-CA), NNE (NNE=DM-(CP+CA+EE+CC)), HCL (HCL=NDF-ADF) and CL (CL=ADF-ADL) values in manure were calculated. Finally based on the results of analysis such as consumed feed ration and nutrient analysis in manure, in vivo nutrients digestibility (%) of rations were found. Also, during last 6 day of the collecting manure period individual daily water consumptions of lambs were determined.

Rumen Parameters

Following the process of collecting the residual feed and manure at the last day of fattening, last live weighting was measured and rumen liquid was extracted by rumen catheter from all lambs. During this period mangers were left empty and animals were allowed to drink water. Measurements of pH in rumen liquid was performed by automatic pH meter (ADWA AD12) and rumen liquid was collected in 3 different sample bottles which were taken from each animal. Those sample bottles were kept in dry ice cabinet at -20°C until analyses.

Gas chromatography was used for the determination of volatile fatty acids (acetic acid, propionic acid, butyric acid, iso butyric acid, valeric acid, isovaleric acid) (Playne 1985, Zdunczyk *et al.* 2013). Samples were first melt at 4°C and then centrifuged at 4000 rpm for 15 minutes. After that, 1 ml of supernatant was taken into 1.5 ml volume capped tube and then 0.2 ml %25 metaphosphoric acid solution was added. Tubes were kept on ice for 30 minutes in order to provide protein precipitation and then centrifuged at 11000 rpm at +4°C again. Carrier gas was Helium (He) and column temperature was programmed to increase from 120°C up to 160°C gradually during the analysis. Before the analysis, standards were prepared with Volatile Free Acid Mix. 46975-U (10 mmol / L) and 1 microlitre rumen liquid taken by means of 10 microlitre injector were injected into gas chromatography device (Shimadzu GC-2010). Temperature of injection block was set to 230°C, FID (Flame ionization detector) temperature of detector was set to 250°C, dry air and hydrogen gas pressure was set to 0.5 kg/cm^3 and analysis was performed by using capillary column.

For ammonia (NH_3) analysis, samples were thawed at 4°C then centrifuged at 14000 rpm at 4° for 15 minutes. After centrifugal process concentration of NH_3-N was marked with indophenol blue method by using UV/Visible spectrophotometer device (Chaney and Marbaeh 1962). In this method ammonia and phenol sodium were oxidised with sodium nitroprusside and a blue colour complex was created. The intensity of blue colour is directly proportional to the concentration of NH_3-N in the sample.

Carcass Parameters

At the end fattening period, all lambs were slaughtered and weighed for their slaughter weights (kg), after the extraction of internal organs they were weighed for hot carcass weights (kg) and then the hot carcasses were kept at +4°C for 24 hours in order to note the cold carcass weight (kg) values. Extracted organs were weighed and marked separately in grams. Based on values of slaughter and hot and cold carcass weights hot and cold carcass efficiency (%) values were calculated using the below formula (Rentfrow 2010).

Hot carcass efficiency (%): (Hot carcass weight / Slaughter weight)x100

Cold carcass efficiency (%): (Cold carcass weight / Slaughter weight)x100

Statistical Analysis

Data were analysed using Variant Analysis Technique based on factorial design random testing. (Duzgunes *et al.* 1987). Different groups were determined by using Duncan Multiple Comparison Test (Duncan 1955). Variants analysis and Duncan Tests SPSS 15 were carried out by running statistical package programmes (Anonymous 2006).

Results

Temperature and Humidity

Outdoor and indoor temperature and humidity values during different fattening periods of closed type sheep barn where fattening was carried out and throughout the fattening periods were given in Table 2. As shown in the table from the early period of fattening minimum and maximum temperatures of inside and accordingly outside of sheep barn were quite low in period transitions (P<0.001), barn interior temperature difference between day and night was minimum in the early period and reached maximum in the late period (P<0.05). Also, significant variations were determined at maximum and minimum humidity rates of sheep barn during the fattening periods.

Fattening Performance

At the beginning of the research, performance parameters of lambs in 2-week periods and throughout fattening are given in Table 3. Live weight (LW), feed consumption (FC), live weight gain (LWG) and feed convertion ratio (FCR) were not affected by the treatment (P>0.05), while feedxadditive interaction was found meaningful in terms of LWG (P=0.001) between groups in the 6th week of fattening period, lambs fed with MH exhibited more LWG than lambs fed with WS.

Table 2. Average values of in and out ambient temperature (°C) and humidity in during and different fattening periods (%)

PROPERTIES	PERIODS					
	0-2 week	2-4 week	4-6 week	6-8 week	0-8 week	P
Out Ambient Temperature						
Minimum	14.5Aa	12.8Aab	11.1Bb	3.7C	10.5	* **
Maximum	23.9A	21.8A	16.5B	9.9C	18.0	* **
Average	20.5Aa	18.5Aa	14.4Bb	7.2C	15.2	* **
Min.-Max. Difference	9.4a	9.0ac	5.4bd	6.2cd	7.5	* **
In Ambient Temperature						
Minimum	21.6A	17.9B	14.3C	7.0D	15.2	* **
Maximum	24.0Aa	21.9Aa	18.1Bb	12.3C	19.1	* **
Average	23.2Aa	20.2Bb	16.8C	10.3D	17.6	* **
Min.-Max. Difference	2.5a	4.0	3.8	5.3b	3.9	* **
In Ambient Humidity						
Minimum	63.7Aa	74.3bd	75.4Bb	67.2ad	70.2	* **
Maximum	71.3A	81.7B	80.5Ba	72.3Ab	76.5	* **
Average	67.2Aa	77.6Bb	77.0b	69.6a	72.8	* **
Min.-Max. Difference	7.6	7.3	5.1	5.1	6.3	

***** Difference among the averages shown with different lower case on the same line are significant (P < 0.05).
** Difference among the averages shown with different upper case on the same line are significant (P ≤ 0.001)

Digestibility of Nutrients and Water Consumption

Averages of nutrient digestibility of rations (DM, OM, CP, CC, EE, CA, NNE, NDF, ADF, HCL and CL) are given in Table 4. Accordingly, differences between roughage sources in terms of digestibility of ration CC were found significant (P<0.05), roughage containing additive had no effect on the digestibility of ration CC (P>0.05). It is observed that ration CC was digested better by lambs consuming ration with MH than lambs consuming ration with WS (P<0.05) and highest digestion levels were achieved with lambs fed with WS+IYM. Additives increased the digestibility of ADF in all groups consuming roughage (P<0.01), while ration cellulose digestibility increased in the group consuming feed with additive in WS (P<0.001). While the differences between roughage sources in terms of HCL digestibility of ration were found significant (P<0.05), additive in roughage had no effect on the digestibility of HCL ration (P>0.05). Lambs fed with ration containing AH had higher rate of HCL digestibility of ration than the group consuming MH **(**P<0.05). By the last day of fattening no difference was not noted in terms of water consumption among the groups (P>0.05) (Table 4).

Table 3. Averages of performance parameters of Anatolian Merino lambs

Properties	TRIAL GROUPS						P VALUES		
	AH	MH	WS	AH+IYM	MH+IYM	WS+IYM	feed	additive	Feed x additive
LW, kg									
Beginnig	26.150	26.016	26.033	26.685	26.175	26.300	0.923	0.712	0.840
0-2 week	30.750	30.816	31.083	32.085	30.900	31.633	0.640	0.401	0.855
2-4 week	34.850	34.183	34.966	35.500	34.200	35.183	0.715	0.717	0.691
4-6 week	38.475	38.733	38.166	38.728	37.800	39.183	0.981	0.293	0.801
6-8 week	41.550	41.866	41.316	41.785	40.475	42.450	0.935	0.094	0.400
LWG, kg									
0-2 week	4.600	4.950	4.800	5.371	4.925	5.334	0.613	0.161	0.951
2-4 week	4.100	3.267	3.367	3.500	4.075	3.550	0.436	0.115	0.262
4-6 week	3.625ab	3.850ab	4.550b	3.257ab	2.575a	4.000 b	0.190	0.672	0.001*
6-8 week	3.075	3.134	3.134	2.700	3.325	3.267	0.651	0.675	0.814
0-8 week	15.400	15.200	15.850	14.828	14.900	16.150	0.923	0.984	0.151
FC, kg/day									
0-2 week	1030.28	994.98	1057.23	1059.66	1011.89	1077.20	0.305	0.663	0.286
2-4 week	1482.97	1426.54	1427.13	1416.63	1454.94	1493.83	0.154	0.681	0.270
4-6 week	1897.62	1828.47	1875.02	1867.15	1881.60	1981.14	0.322	0.847	0.240
6-8 week	1939.19	1785.58	1819.90	1819.54	1844.26	1956.50	0.316	0.753	0.076
0-8 week	1587.52	1546.66	1508.90	1554.48	1521.38	1627.17	0.279	0.134	0.071
LWG, g/day	275	271	283	265	266	289	0.923	0.973	0.151
FCR (0-8 week)	5.775	5.724	5.356	5.931	5.753	5.646	0.850	0.382	0.135

* Difference between the averages shown with different lower case on the same line are significant (P≤0.001).

Table 4. Average values of nutrients digestibility of rations and water consumption of lambs

PROPERTİES	TRIAL GROUPS						P VALUES		
	AH	AH+IYM	MH	MH+IYM	WS	WS+IYM	feed	additive	Feed x additive
Digestibility, %									
DM	75.35	74.08	73.17	73.73	74.08	74.95	0.238	0.933	0.403
OM	77.23	75.85	75.75	75.73	75.72	75.72	0.497	0.614	0.464
CP	81.01	78.12	79.03	78.58	78.51	77.67	0.286	0.060	0.338
CC	52.23ab	48.58ab	45.92b	48.83b	52.68a	53.41a	0.017*	0.998	0.222
EE	89.64	89.70	86.89	89.23	87.31	88.17	0.308	0.305	0.643
CA	56.79	52.41	47.55	54.79	53.94	51.41	0.562	0.967	0.148
NNE	81.10	79.88	80.61	80.13	80.16	80.98	0.965	0.638	0.444
NDF	64.53	61.84	58.81	61.64	61.38	64.13	0.141	0.476	0.184
ADF	40.55a	45.34b	43.03a	48.97b	42.27a	51.17b	0.263	0.002**	0.682
CL	70.87a	78.54ab	74.07ab	79.58ab	70.81a	81.56b	0.583	0.000***	0.449
HCL	73.75b	69.87ab	68.80a	68.19a	69.53ab	69.97ab	0.013*	0.131	0.144
Water consumption, lt									
5. Day	4.3755	4.167	4.134	4.188	4.250	3.817	0.719	0.395	0.670
6. Day	4.87	4.284	4.000	4.828	4.125	4.434	0.673	0.494	0.094
7. Day	4.375	4.084	4.517	4.300	3.800	3.367	0.273	0.950	0.195
8. Day	4.000	3.550	3.834	3.614	3.725	3.700	0.146	0.692	0.133
9. Day	4.450a	4.217a	3.617b	4.386a	4.475a	3.534b	0.487	0.595	0.026**
10. Day	3.375	3.300	3.034	3.286	3.200	3.250	0.326	0.444	0.371

Difference among the averages shown with different letters on the same line are significant *(P<0.05) **(P<0.01) ***(P<0.001).

Table 5. Average values of rumen parameters obtained from trial groups (Mmol/lt)

PROPERTİES	TRIAL GROUPS						P VALUES		
	AH	AH+IYM	MH	MH+IYM	WS	WS+IYM	feed	additive	Feed x additive
Acetic acid	27.41a	28.73ab	33.04ac	28.10b	26.96a	33.90c	0.320	0.457	0.008**
Propionic acid	13.17ab	9.96a	15.05b	9.93a	11.51ab	15.15b	0.353	0.108	0.002**
Butyric acid	8.10	9.43	10.23	9.86	7.70	9.40	0.333	0.343	0.598
İsobutyric acid	1.11	1.22	1.19	1.18	1.15	1.23	0.954	0.385	0.699
Valeric acid	1.38	1.41	1.47	1.43	1.34	1.77	0.573	0.249	0.256
İsovaleric acid	1.23	1.33	1.33	1.27	1.25	1.39	0.961	0.499	0.672
TVFA	52.41a	52.09a	62.31b	51.78a	49.91a	62.84b	0.331	0.804	0.006**
A/P	2.11a	2.92b	2.26a	2.93b	2.37a	2.31a	0.515	0.015*	0.145
Ammonia	1.78a	3.45a	4.09b	4.11b	2.87a	2.36a	0.030*	0.455	0.242
pH	7.12	7.17	6.98	7.08	7.05	6.97	0.607	0.861	0.793

Differences among the averages shown with different letters on the same line are significant. *(P<0.05), **(P<0.01).

Rumen Parameters

Feed x additive interaction was found significant among the groups in terms of acetic acid and propionic acid (P<0.01). Additive agent decreased the rumen acetic and propionic acid molar concentration in the group consuming MH, whereas it increased the acetic acid molar concentration in the group consuming WS (P<0.05) (Table 5). Effects of roughage type and additive agent on the concentrations of butyric acid, isobutyric acid, valeric acid and isovaleric acid were found similar (P>0.05). Feed x additive interaction between groups was found significant in terms of Total Volatile Fatty Acid (TVFA) (P<0.01). While the IYM did not affect the amount of rumen TVFA in the groups consuming AH (P>0.05) it caused decrease in the groups consuming MH (P<0.01) and caused increase in the groups consuming WS (P<0.01). For lambs fed with rations containing MH and WS+IYM, rumen TVFA molar concentration was found similar and significantly higher than the other groups (P<0.01). Additive agent in AH and MH increased the rate of rumen acetic acid/propionic acid (A/P) (P<0.05). In the group of lambs feeding with MH ratio of rumen to ammonia was higher than the other groups (P<0.05) but no difference was seen among groups in terms of ruminal pH values (P>0.05).

Carcass Parameters and Internal Organ Weights

Carcass parameters (hot and cold carcass weights, their yields and slaughter weights) and weights of some internal organs (spleen, liver and lung) were not affected from roughage or roughage containing IYM (P>0.05), but weight of heart was increased with additive agent in AH (P<0.05) (Table 6).

Table 6. Averages of carcass parameters and weights of some internal organs according to the trial on groups.

Properties	TRIAL GROUPS						P VALUES		
	AY	AY+IYM	MH	MH+IYM	WS	WS+IYM	feed	additive	Feed x additive
Slaughter and carcass parameters*									
HCW, kg	18.35	18.80	18.53	18.91	18.40	19.37	0.902	0.267	0.888
CCW, kg	18.05	18.44	18.20	18.63	18.05	19.00	0.916	0.278	0.895
SW, kg	41.55	41.87	41.32	41.78	40.47	42.45	0.935	0.094	0.400
HCY, %	44.03	44.81	44.91	45.30	45.42	45.62	0.687	0.651	0.973
CCY, %	43.31	43.94	44.10	44.61	44.56	44.76	0.711	0.662	0.986
Internal organ weights, g									
Spleen	173.50	170.50	172.84	176.71	157.50	161.34	0.261	0.844	0.923
Liver	862.50	892.67	891.00	870.43	859.25	861.17	0.853	0.904	0.800
Lung	670.00	673.00	637.34	660.86	648.50	640.00	0.757	0.846	0.904
Heart	174.25a	195.50b	184.34ab	189.14ab	182.50ab	198.84b	0.184**	0.033	0.328

*HCW: Hot Carcass Weight, CCW: Cold Carcass Weight, SW: Slaughter Weight, HCY: Hot Carcass Yield, CCY: Cold Carcass Yield
** Difference between the averages shown with different letters on the same line are significant statistically (P<0.05).

Discussion

It is emphasized that beneficial effects of yeast, one of the feed additive agents as a stimulant for growing in the breeding industry are related with their increasing effect on the performance of ruminants (Abd-El Ghani 2004, Moharrery and Asadi 2009, Ezema 2013). In addition, adding inactivated yeast metabolite to the rations prepared with different roughage did not make any difference in the parameters of fattening performance (LW, LWG, FC, FCR) of Anatolia Merinos lambs. Apart from these researchers (Arcos-Garcia *et al.* 2000, Dolezal *et al.* 2005, Pina *et al.* 2006, Gomes *et al.* 2014) reporting the effects of yeast are related with composition of ration and the amount of yeast used in ration, some researchers (Pina *et al.* 2006, Gomes *et al.* 2014) reported that the effect is related with limiting the daily amount of roughage in the group of lambs nourished with intense feed. In this study the amount of daily roughage fed to lambs for cellulolytic activity was kept at the required level and constant during the trial. On the other hand, during the trial period (September-November) it was determined that variations of temperature and humidity during the fattening periods were significant. This indicates the parameters of fattening performance could be affected from the environmental factors such as temperature and humidity. Researchers alreadyreport (Kocaman *et al.* 2007, Yaganoglu 2011, Goncu *et al.* 2015, Niyas *et al.* 2015) that ambient temperature and humidity are significant environmental factors during the periods of growing and development, so in cases of ambient temperature decreases, the energy sourced by feed consumption is utilised in order to hold that temperature constant in connection with lambs' energy requirement increase and thus affecting the performance of fattening. These reports support the results of our study.

It is determined that CC and digestibility of HCL was affected by the type of roughage in ration ($P<0.05$), additive agent increased digestibility of ADF in all groups fed by roughage ($P<0.01$) and increased the digestibility of CL in the group fed with WS ($P<0.001$). While no relationship between the consumption of roughage with low cell wall components and the rate of digestible nutrient was observed, the consumption of roughage with high cell wall components had significant relation with the ratio of digestible nutrients and it is noted that this is the result of different levels of digestible energy provided from cell wall components (Sehu *et al.* 1998). It is specified that with the usage of IYM additive, more benefit can be provided from organic indigestible carbohydrate and other organic/inorganic factors, improvement on the digestive levels of feed would be observed (Grochowska *et al.* 2012, Rufino et al 2013). It is also specified that limited feeding, roughage: concentrate feed ratio and feed consumption, intensity of anaerobic and cellulolytic bacteria are effective factors; and in connection the digestibility of cellulose increases (Lima *et al.* 2012). Some researchers associated the effects of IYM with the low-quality roughage containing high CL in ration (Elseed and Abusamra 2007, Tang *et al.* 2008, Tripathi and Karim 2011 Turkmen *et al.* 2011, Zain *et al.* 2011). According to the results of this research, digestibility of other nutrients were not affected by the addition of IYM ($P>0.05$). It is stated that the digestibility of organic material is not affected by yeast addition and this may be caused by the digestion of yeast cell produced metabolites by rumen microorganisms (Oeztuerk 2009).

No difference was determined among the trial groups in terms of water consumption. To date, no literature exists on the water consumption of Anatolian Merinos lambs fed with IYM additives; however, it is indicated that daily water consumption of lambs vary between 3.05 - 5.7 liters (Schoeman and Visse 1995, Parker and Brown 2003). Findings regarding water consumption in this study (3.03 - 3.38 lt/day) are in line with literature.

Inactivated yeast metabolite additives decreased the rates of rumen acetic acid, propionic acid and TVFA in MH while increasing the rates of acetic acid and TVFA **in** WS ($P<0.01$). It is stated that organic carbohydrates of acetic acid may be changed depending on the fermentation of cellulolytic bacteria (Opsi *et al.* 2012) and rate of propionic acid decrease significantly with cereal feeding (Lila *et al.* 2004, Hill *et al.* 2009). It is noted that concentration of total volatile fatty acid increased according to the density of bacteria in rumen and the realisation of anaerobic rumen ambiance (Arcos-Garcia *et al.* 2000, Alshaikh *et al.* 2002, Dolezal *et al.* 2005, Kowalik *et al.* 2015); however it is emphasised that it may decrease depending on the type or quality of yeast used (Angeles *et al.* 1998, Thrune *et al.* 2009, Tripathi and Karim 2011). It is stated that yeasts contribute to the maintenance of rumen pH due to similar reasons (Ghoneem and Mahmoud 2014). In this study rumen pH was also maintained. The concentration of ammonia were influenced by the type of roughage and that ruminal concentration of ammonia increased in lambs fed with MH ($P<0.05$) in our study. This result was associated with the fact that MH contains more soluble protein than other roughage types. This is because it is expressed as an expected case that IYM addition would cause an increase in bacteria intensity in rumen and the amount of microbial nitrogen transferring to small intestine and thus ammonia nitrogen would transform into microbial protein more efficiently (Denev *et al.* 2007, Patra 2012). It is also indicated that the increase in ammonia and TVFA was provided for with the addition of IYM ration containing % 49:51 roughage:strong feed (Jurkovich *et al.* 2014) and that the

concentration of rumen ammonia varies due to the type of the yeast used (Arcos-Garcia *et al.* 2000).Slaughter weight, hot and cold carcass weights and yield; weights of spleen, liver and lung were not affected by roughage and additive matter (P>0.05); however additive agent increased the heart weights of lambs fed with AH (P<0.05). These results were found similar with the results of studies stating that carcass parameters are not affected from addition of yeast (Kawas *et al.* 2007, Rufino *et al.* 2013).

Conclusion

Lamb fattening during the off-mating season, seasonal temperature and humidity variations were a significant factor on the performance; IYM additives in lamb rations had positive effects on some cell wall components digestibility and rumen fermentation; each of the as a rich roughage AH, as a medium quality roughage MH or as a low quality roughage WS might be used as roughage material during lamb fattening based on intensive feed; and that further studies where higher rations of roughage are used in order to manifest the effects of IYM more clearly.

Acknowledgements

Authors thanks to Ankara University Scientific Research Projects Coordination Unit for their support. (This work was supported by Ankara University Scientific Research Projects Coordination, grant number: 15L0447004)

References

Abd-El Ghani, A. A. (2004). Influence of diet supplementation with yeast culture (*Saccharomyces cerevisiae*) on performance of Zaraibi goats. Small Rum. Res. 52: 223-229.

Akyıldız, A. R. (1984). Yemler bilgisi laboratuvar kılavuzu. Ankara Üniversitesi Zir. Fak. Yay. 286s. Ankara.

Alshaikh, M. A., Alsiadi, M. Y., Zahran. S. M., Mogawer, H. H., & Aalshowime, T. A. (2002). Effect of feeding yeast culture from different sources on the performance of lactating holstein cow in Saudi Arabia. Asian-Australas. J. Anim. Sci. 15: 352–356.

Angeles, C., Mendoza, M. G. D., Cobos, P. M. A., Crosby, G. M. M., & Castrejon, P. F. A. (1998). Comparison of commercial yeast culture (*Saccharomyces cerevisiae*) on ruminal fermentation and digestion in sheep fed on corn stover diet. Small Rum. Res. 31: 45-50.

Anonymous. (2014a). www.suthayvanciligi.com/urunlerimiz/ diamond-v

Anonymous. (2014b). www.tempe.com.tr

Anonymous. (2015). www.diamondv.com/wp-content/ uploads/ product profile_xp_ls.pdf.

Anonymous. (2016). htttps://intweb. tse.org.tr/standard. TS No: TS9610 Hayvan yemleri- metabolik (çevrilebilir) enerji tayini kimyasal metod. Kabul tarihi: 03.12.1991

Anonymous. (2006). SPSS 15.0 Command syntax reference. SPSS Inc Copyright © 2006 by SPSS Inc. Chicago, IL 60606-6412 www.spss.com.

Arcos-Garcia, J. L., Castrejon, F. A., Mendoza, G. D., & Perez Gavilan, E. P. (2000). Effect of two commercial yeast cultures with *Saccharomyces cerevisiae* on ruminal fermentation and digestion in sheep fed sugar cane tops. Livest Prod. Sci. 63: 153-157.

AOAC. (1984). Official methods of analysis (14th Ed.) association of official analytical chemists. Washington. DC. p:1112.

Chaney, A. L., & Marbaeh, E. P. (1962). Modified reagents for determination of urea and ammonia. Clin. Chem. 8: 130-132.

Dağsöz, A. K., & Bayraktar, K. G. (1999). Türkiye'de derece-gün sayıları ve enerji politikamız. yapıda yalıtım konferansı bildiriler kitabı. İstanbul.

Denev, S. A., Peeva, T., Radulova, P., Stancheva, N., Staykova, G., Beev, G., Todorova, P., Tchobanova, S. (2007). Yeast cultures in ruminant nutrition. Bulg. J. Agric. Sci. 13: 357-374.

Dolezal, P., Dolezal, J., & Trinacty, J. (2005). The effect of *Saccharomyces cerevisiae* on ruminal fermentation in dairy cows. Czech J. Anim. Sci.50: 503-510.

Düzgüneş, O., Kesici, T., & Gürbüz, F. (1987). İstatistik metodları-I. A.Ü. Zir. Fak. Yay. 861. Ders Kitabı: 299. 218 s. A.Ü. Basımevi. Ankara.

Duncan, D. B. (1955). Multiple range and multiple f test. Biometrics 11: 1-42.

Elseed, F., & Abusamra, M. A. (2007). Effects of supplemental yeast (*Saccharomyces cerevisiae*) culture on NDF digestibility and rumen fermentation of forage sorghum hay in Nubian goat's kids. Res. J. Agric. & Biol. Sci. 3(3):133-137.

Ezema, C. (2013). Probiotics in animal production: A review. J. Vet. Med. Anim. Health 5(11): 308-316.

Ghoneem, W. M. A., & Mahmoud, A. E. M. (2014). Effect of inactivated and dried yeast on productive performance of Barki lambs. Asian J. Anim. Vet. Adv. ©2014 Academic J. Inc.

Gomes, L. C., Alcalde, C. R., Lima, L. R., Lima, L. S., Souza, R., & Possamai, A. P. S. (2014). Nutritive value of diets containing inactive dry yeast for lactating Saanen goats. R. Bras. Zootec. 43(1):36-43.

Göncü, S., Önder, D., Koluman, N., & Mevliyaoğulları, E. (2015). Sıcak ve nemli koşullara uygun hayvan barınak özellikleri. Tüsedad 5(30): 42-47.

Grochowska, S., Nowak, W., Mikuła, R., & Kasprowicz-Potocka, M. (2012). The Effect of *Saccharomyces cerevisiae* on ruminal fermentation in sheep fed high- or low-NDF rations. J. Anim. Feed Sci. 21: 276–284.

Haddad, S. G., & Goussous, S. N. (2005). Effect of yeast culture supplementation on nutrient intake, digestibility and growth performance of Awassi lambs. Anim. Feed. Sci. Technol. 118: 343-348.

Hill, S. R., Hopkins, B. A., & Davidson, S. (2009). The addition of cottonseed hulls to the starter and supplementation of live yeast or mannanoligosaccharide in the milk for young calves. J. Dairy Sci. 92: 790-798.

Inge, N. A., Bogaert, V., & Maeseneire, L. D. (2009). Extracellular polysaccharides produced by yeast and yeast-like fungi. yeast biotechnology: Diversity App. 651-671.

Jurkovich, V., Brydl, E., Kutasi, J., Harnos, A., Kovacs, P., Könyves L., Muravölgyi, & Febel, H. (2014). The effects of *Saccharomyces cerevisiae* strains on the rumen fermentation in sheep fed with diets of different forage to concentrate ratios. J. Appl. Anim. Res. 42 (4): 481-486.

Karademir, G., & Karademir, B. (2003). Yem katkı maddesi olarak kullanılan biyoteknolojik ürünler. Lalahan Hay. Araşt. Enst. Derg. 43: 61-74.

Kawas, J. R., García-Castillo, F., Garza-Cazares, H., FimbresDurazo, Olivares-Sáenz, E., Hernández-Vidal, G., & Lu, C. D. (2007). Effects of sodium bicarbonate and yeast on productive performance and carcass characteristics of light-weight lambs fed finishing diets. Small Rum. Res. 67: 157-163.

Kocaman, İ., Konukçu, F., & İstanbulluoğlu, A. (2007). Hayvan barınaklarında ısı ve nem dengesi. KSÜ Fen & Müh. Derg. 10(1): 137-140.

Kocaoğlu, G. B., & Kara, K. (2010)**.** Ruminant beslemede alternatif yem katkı maddelerinin kullanımı: 2. organik asit, yağ asiti, adsorban. Erciyes Üniv. Vet. Fak. Derg. 7: 43-52.

Kowalık, B., Skomial, J., Miltko, R., & Majewska, M. (2015). The effect of live *Saccharomyces cerevisiae* yeast in the diet of rams on the digestibility of nutrients, nitrogen and mineral retention and blood serum biochemical parameters. Turk J. Vet. Anim. Sci. (2016) 40: © TÜBİTAK.

Lila, Z. A., Mohammed, N., Yasui, T., Kurokawa, Y., Kanda, S., & Itabashi, H. (2004). Effects of twin strain of *Saccharomyces cerevisiae* live cells on mixed ruminal microorganism fermentation in vitro. J. Anim. Sci. 82: 1847–1854.

Lima, L. S., Alcalde, C. R., Gomes, L. C., Souza, R., Rigolon, L. P., & Nakamura, C. V. (2012). Effect of inactive dry yeast from sugar cane as protein source on rumen fermentation in saanen goats. Arg. Bras. Med. Vet. Zootec. 64(1): 145-154.

Moharrery, A., & Asadi, E. (2009). Effects of supplementing malate and yeast culture (*Saccharomyces cerevisiae*) on the rumen enzyme profile and growth performance of lambs. J. Anim. Feed Sci. 18: 283-295.

Niyas, P. A., Chaidanya, K., Shaji, S., Sejian, V., Bhatta, R., Bagath, M., Rao, G., Kurien, E., & Girish, V. (2015). Adaptation of livestock to environmental challenges. J. Vet. Sci. Med. Diagn. 4: 3.

NRC. (2007). Nutrient requirements of small ruminants. Animal Nutrition Series. National Research Council. Washington. D.C.

Oeztuerk, H. (2009). Effects of live and autoclaved yeast cultures on ruminal fermentation in vitro. J. Anim. Feed. Sci. 18: 142–150.

Opsi, F., Fortina, R., Tassone, S., Bodas, R., & Lopez, S. (2012). Effects of inactivated and live cells of *Saccharomyces cerevisiae* on in vitro ruminal fermentation of diets with different forage: concentrate ratio. J. Agr. Sci. 150: 271–283.

Parker, D. B., & Brown, M. S. (2003). Water consumption for livestock and poultry production. Encyclopaedia of Water Sci. 1-5.

Patra, A. K. (2012). The use of live yeast products as microbial feed additives in ruminant nutrition. Asian J. Anim. Vet. Adv. 7(5): 366-375.

Pina, D. S., Valadares Filho, S. C., Valadares, R. F. D., Campos, J. M. S., Detmann, E., Marcondes, M. I., Oliveira, A. S., & Teixeira, R. M. A. (2006). Consumo e digestibilidade aparente total dos nutrientes. produção e composição do leite de vacas alimentadas com dietas contendo diferentes fontes de proteína. R. Bras. Zootec. 35:1543-1551.

Playne, M. J. (1985). Determination on ethanol volatile fatty acids, lactic and succinic acids in fermentatıon liquids by gas chromatography. J. Sci. Food Agr. 36(6): 638-644.

Rentfrow, G. (2010). How much meat to expect from a carcass. Anim. Food Sci. University of Kentucky College of Agriculture, Lexington, KY, 40546 ASC-179.

Rufino, L. D. A., Pereira, O. G., Ribeiro, K. G., & Valadares Filho, S. C. (2013). Effect of substitution of soybean meal for inactive dry yeast on diet digestibility, lamb's growth and meat quality. Small Rum. Res. 111: 56-62.

Schoeman, S. J., & Visse, J. A. (1995). Water intake and consumption in sheep differing in growth potential and adaptability. S. Afr. J. Anim. Sci. 25(2): 75-79.

Şehu, A., Yalçın, S., & Önol, A. G. (1998). Kaba yemlerin bazı özelliklerinden yararlanarak kuzularda kuru madde tüketimi ve canlı ağırlık artışının belirlenmesi. Turk J. Vet. Anim. Sci. 22: 475-483©TÜBİTAK.

Tang, S. X., Tayo, G. O., Tan, Z. L., Sun, Z. H., Shen, L. X., Zhou, C. S., Xiao, W. J., Ren, G. P., Han, X. F., & Shen, S. B. (2008). Effects of yeast culture and fibrolytic enzyme supplementation on in vitro fermentation characteristics of low-quality cereal straws. J. Anim. Sci. 86: 1164-1172.

Thrune, M., Bach, A., Ruiz-Moreno, M., Stern M. D., Linn, & J. G. (2009). Effects of *Saccharomyces cerevisiae* on ruminal pH and microbial fermentation in dairy cows: yeast supplementation on rumen fermentation. Livestock Sci. 124(1): 261–265.

Tripathi, M. K., & Karim, S. A. (2011). Effect of yeast cultures supplementation on live weight change, rumen fermentation, ciliate protozoa population, microbial hydrolytic enzymes status and slaughtering performance of growing lamb. Livestock Sci. 135: 17-25.

Türkmen, İ. İ., Biricik, H., Deniz, G., Gezen, Ş. Ş., Tuncer, Ş. D., Çolpan, İ., Küçükersan, M. K., Küçükersan, S., Yalçın, S., Şehu, A., Saçaklı, P., Ergün, A., & Yıldız, G. (2011). Temel yem bilgisi ve hayvan besleme. Anadolu Ünv. Yay. 70-88, 1. Baskı. Eskişehir, Turkey.

Van Soest, P. J. (1994). Fiber and physicochemical properties of feeds in: nutritional ecology of the ruminant. Cornell University press. pp. 140-155.

Vyas, D., Uwizeye, A., Mohammed, R., Yang, W. Z., Walker, N. D., & Beauchemin, K. A. (2014). The effects of active dried and killed dried yeast on subacute ruminal acidosis, ruminal fermentation and nutrient digestibility in beef heifers. J. Anim. Sci. 92: 724–732.

Yağanoğlu, A. V. (2011). Hayvan barınaklarında çevre koşullarının düzenlenmesi. Atatürk Üniv. Zir. Fak. Derg. 17(1): 121-129.

Zain, M., Jamarun, N., Arnim, A., Ningrat, R.W.S., & Herawati, R. (2011). Effect of yeast (*Saccharomyce cerevisiae*) on fermentability, microbial population and digestibility of low quality roughage in vitro. Archiva Zootech. 14(4): 51-58.

Zdunczyk, Z., Gruzauskas, R., Juskiewicz, J., Dauksiene, A., Raceviciute-Stupeliene, A., & Jarule, V. (2013). Effect of dıetary probiotic, prebiotic mannanoligosaccharides and their combination on caecal parameters in hens. Vet. Med. Zoot. 63 (85).

Livestock Studies, 2020; 60 (2): 46-53
http://doi.org/10.46897/livestockstudies.846408

RESEARCH PAPER

LIVESTOCK STUDIES

Use of multiple regression modeling for the evaluation of lactation characteristics of Awassi sheep

İrfan Güngör[1*], Fatih Atasoy[2]

[1] General Directorate of Agricultural Research and Policies, Ankara, Turkey.
[2] Ankara University, Faculty of Veterinary Medicine, Department of Animal Husbandry and Animal Nutrition, Ankara, Turkey.

Article History
Received: 23 September 2020
Accepted: 13 November 2020
First Online: 25 December 2020

Corresponding Author
Tel.: +903123157622
Email: irfan.gungor@tarimorman.gov.tr

Keywords
Awassi sheep
Lactation curve
LEG4
Milk yield

Abstract

This study was conducted to identify the best model characterizing the lactation curves of Awassi sheep, and to calculate the estimated lactation milk yield (ELMY) per sheep based on the model parameters. The study also investigated the factors affecting the observed lactation milk yield (OLMY) and ELMY, as well as the identified model parameters. OLMY was 111.47±3.504 kg, with a lactation period of 133.70±1.714. In an analysis of the HO, R^2 and r parameters together, the fourth-order Legendre polynomial (LEG4) model was found to best predict the lactation curves of the Awassi sheep in the present study.

Introduction

Sheep provide many economically important products, including mutton, milk, and fleece (Güngör and Akçapınar, 2013). In regions with considerable pasture and arid climates, raising sheep is the leading animal husbandry activity (Güngör and Akçapınar, 2013). As of 2019, there were a total of 37.276.050 head of sheep being raised in Turkey (TUIK, 2019), with Awassi is one of the most widely bred sheep breeds. Awassi is a fat-tailed species of dairy sheep, named after the tribe of Al Awas that originates from the area between the Tigris and Euphrates rivers (Epstein, 1982). Awassi are kept in flocks, and raised in large numbers in the Şanlıurfa, Gaziantep, Kilis and Hatay provinces of Turkey and their vicinities, that is to say, in the Southeastern Anatolia region (TAGEM, 2009). They are considered to be superior to other dairy species due to their ability to adapt to different environments and their strong herd instinct (TAGEM, 2009).

Close to 31,473 tonnes of sheep milk is produced in Turkey (TUIK, 2019), being used predominantly in cheese production, and this has led sheep farming to gain importance in recent years (Çoban *et al*, 2013), resulting in breeding efforts aimed at improving milk yield. Conventional farming methods should be used for efficient production and the full utilization of the flock's potential, with one such method being the use of the lactation curve model within an optimum raising-feeding strategy, depending on the needs and potential of the flock. Using the lactation curve for breeding, flock management and production planning is reported to help increase efficiency in sheep husbandry (Montaldo *et al*., 1997; Tzouramani *et al*., 2011; Angeles-Hernandez, 2013). Lactation models have traditionally been developed initially for cattle (Morant and Gnanasakthy, 1989; Beever *et al*., 1991; Tekerli *et al*., 2000), while the number of models used for the characterization of the lactation curves of sheep, on the other hand, has been limited (Sakul and Boylan, 1992; Dağ *et al*., 2005; Keskin and Dağ, 2006).

In lactation curve studies, the goal is to create a lactation curve based on the milk yield of animals milked at different times, and to identify the environmental factors and values that affect the curve. Using the lactation curve, it is possible to understand the effects of such factors as species, lambing year, lambing season, lactation order and age of first lactation on both the lactation

curve and the total milk yield (Gipson and Grossman, 1990). Lactation curve coefficients are used to examine the effects of the lactation curve on milk production and economic factors (Grossman *et al*., 1986), determining the shape and the slope of the curve. The sizes of these coefficients, or in other words, the shape of the lactation curve is affected by factors such as the number of births, lambing season, lactation order, age of first lambing, care-feeding, health status and genotype. The effects of these factors can vary by flock or by years, and this is why the parameters of a lactation curve identified for a particular flock can be said to be unique to that flock (Tekerli *et al*., 2000).

The most well-known lactation curve model still in use is the one proposed by Wood in 1967 (Wood, 1967). The Wood lactation model is used widely in studies examining the factors affecting the lactation curve (Yılmaz *et al*., 2004), while many new models have been proposed since 1967. The Wood lactation model has been used previously in studies of Awassi sheep (Yılmaz *et al*., 2004). Many parametric models have been developed to characterize lactation curves, including inverse polynomial, gamma type and exponential functions. With the increased use of individual records, more flexible functions for the making of linear predictions have started to be used more frequently (Prakash *et al*., 2016).

Selection efficiency could be increases through the use of the right lactation curve model, the right milk yield estimate and the right selection of animals, and identifying the lactation curve would help with efficient breeding. This study aims to identify the model with the best fit for the lactation curves of Awassi sheep. The lactation curve was created using the model proposed in this study, and the environmental factors that affect parameters of the lactation curve and OLMY were examined.

Materials and Method

Animal Material

The study was conducted between 2013 and 2015 on Awassi sheep at the Şanlıurfa GAP Agricultural Research Institute of the General Directorate of Agricultural Research and Policies of the Ministry of Agriculture and Forestry, making use of the data from 287 sheep (year 2013, n=101; year 2014, n=82; year 2015, n=104) raised within an Awassi sheep breeding project. The lactation milk yields and lactation curves of ewes that lambed in different periods were examined. Lambing started in a different month each year, in November, December and January. The flock was cared for and fed following routine procedures at the Institute. Milk controls were performed on a 24-hour basis, and repeated every 20 days. Milk controls ended when two-thirds of the flock finished lactating, upon which milking was ended for the entire flock. The lactation curve was created based on 1,136 daily milk yield records from 213 ewes. Ewes with fewer than four control milk yields in a lactation period were excluded from the analysis.

Statistical Analysis

Table 1 presents the lactation models used in the study. The data analysis was carried out using the PROC NLIN option in the SAS (2000) software package. The model with the best fit was identified through a comparison that took into account the correlation coefficient (r), average (residual) error (AE) and coefficient of determination (R^2). After identifying the model that best characterized the lactation curve, the model parameters were used to calculate estimated lactation milk yields (ELMY).

PROC NLIN procedure:

PROC NLIN; byanim;

MODEL y = <nunlinear model expression>;

PARMS a=<değer>, b=<değer>, c=...., ,,,;

OUTPUT OUT=<output file name> PREDICTED=<name> RESIDUAL=<residual name>

SSE=<errorsums of square> PARMS= a, b, c, … (*names of model parameterspredicted*);

RUN;

To obtain these estimates, daily and total lactation milk yields were calculated for each ewe. The ELMY values obtained using the parameters of the selected model were compared with the OLMY values calculated using the Fleischmann method.

After calculating OLMY and ELMY, SAS PROC CORR was used to calculate the coefficients of the correlation between OLMY and ELMY and the parameters of the model (LEG4).

The model used to examine the environmental factors that affect the observed lactation milk yield (OLMY), the estimated lactation milk yield (ELMY) and the model parameters was as follows: Y_{ijk}

$$Y_{ijk} = \mu + YA_i + DT_j + b\ (X_{ijk}) + e_{ijk}$$

Y_{ijk}: ELMY, OLMY, model parameters, μ: Overall mean in terms of the analyzed trait, YA_i: i^{th} lambing year-Month, DT_j: j^{th} birth type, b: the partial regression coefficient of X_{ijk}, X_{ijk}: lactation lenght of the k^{th} ewe, e_{ijk}: Residual associated with Yijk

The following model was used to analyze the factors (environmental and flock management) affecting lactation lenght (LD):

$$Y_{ijk} = \mu + YA_i + DT_j + e_{ijk}$$

Y_{ijkl}: ELMY, OLMY, model parameters, μ: Overall mean in terms of analyzed trait, YA_i: ith lambing year-Month, DT_j: jth birth type, e_{ijk}: Residual associated with Yijk

The differences between the levels of environmental factors were tested using the LSMEANS/TUKEY option within the PROC GLM system (SAS, 2000).

Table 1. Lactation model

Model Name	Model	Explanation
Nelder, 1966	$y_t = {}^{t}/_{a} + bt + ct^2$	y_t: milk yield (gr / day) at time t (day) of lactation a, b, c, and d are model parameters that determine the shape and scale of the curve t: Control interval (days)
Wood, 1967	$y_t = at^b e^{-ct}$	y_t: milk yield (gr / day) at time t (day) of lactation a, b, c, and d are model parameters that determine the shape and scale of the curve t: Control interval (days)
Morant and Gnanasakthy, 1989	$y_t = e^{(a - bt_1 - ct_1^2 - d/t)}$	y_t: milk yield (gr / day) at time t (day) of lactation a, b, c, and d are model parameters that determine the shape and scale of the curve t: Control interval (days)
Cobby and Le Du, 1978	$y_t = a - bt - ae^{-ct}$	y_t: milk yield (gr / day) at time t (day) of lactation a, b, c, and d are model parameters that determine the shape and scale of the curve t: Control interval (days)
Legendrepolynomial Kirkpatrick *et al*., 1990; Schaeffer, 2004; Silvestre *et al*., 2006; Koncagül *et al*., 2012		$t_{1=}\ (t - Laktasyon\ uzunluğu/2)/100$ y_t= milk yield on day t control day after birth the value of t varies between -1 and +1 (Equation 1) $t = 2\left(\frac{tg - tg_{min}}{tg_{max} - tg_{min}}\right) - 1$ [1] tg= control day tg_{min} = first control day tg_{max}= last control day
	$y_t = \sum_{i=1}^{n} \alpha_i \Phi(t)$	$\Phi(t) = \sqrt{\frac{2n+1}{2}} P_n(t)$ ϕ(t) =normalized polynomial $P_n(t)$ = n^{th} order of the polynomial
	$P_2(t) = \frac{1}{2}(3t^2 - 1)$	LEG2
	$P_3(t) = \frac{1}{2}(5t^3 - 3t)$	LEG3
	$P_4(t) = \frac{1}{8}(35t^4 - 30t^2 + 3)$	LEG4

Results

Descriptive statistics of the traits are presented in Table 2. A large variation is observed in ewe weight, lactation lenght, lactation milk yield and average daily milk yield. The majority of the flock consisted of young mothers (2, 3 and 4 years old), while there were few older ewes (5 years old or above accounted for 12% of the flock).

Table 3 reports the r, R^2 and AE values obtained from different models by years. The Cobby and Le Du, Nelder and Wood models had the lowest performances in terms of AE. The rest of the models had AE values that were indistinguishable from zero. The R^2 values in the Nelder and Cobby and Le Du models were found to be negative. Legendre polynomials (LEG) had the highest R^2 value. Among the Legendre polynomials, LEG4 had the highest R^2 value (0.99), followed by LEG3 (0.93) and LEG2 (0.88). The values obtained from the other models were smaller.

Table 2. Descriptive statistics for the descripted statistics for BW, EW, LD, OLMY and DMY

Sex	Number (%)	Age	Number (%)		Mean ± St. Dev	Min.	Max.
Male	147 (55,3)	2	79 (29,7)	Birth Weight (kg)	4.512 ± 0.737	2.1	6.8
Female	119 (44,7)	3	79 (29,7)	Ewe Weight (kg)	55.848 ± 7.276	39.2	73.4
Type of birth	Number (%)	4	76 (28,6)	LD (Days)	128.643 ± 29.597	29.0	192.0
Single	205 (77,1)	5	32 (12,0)	OLMY (kg)	105.210 ± 51.103	26.9	288.7
Twins	61 (22,9)	Total	266 (100,0)	Average Daily Milk Yield (g)	803.467 ± 299.493	208.4	1874.5

Table 3. Comparison statistics used for the characterization of the lactation curves of Awassi sheep

Year	Model	n	r	R^2	AE
2013	Wood	59	0.101±0.073	0.175±0.019	621.479±27.917
	Nelder	59	0.572±0.050	-2.190±0.388	1041.79±136.669
	Morant and Gnanastky	59	0.953±0.010	0.994±0.001	0.429±0.314
	Cobby and Le Du	59	0.577±0.047	-3.463±1.773	484.290±174.501
	LEG2	59	0.882±0.015	0.982±0.003	0.000±0.000
	LEG3	59	0.937±0.010	0.992±0.002	0.000±0.000
	LEG4	59	0.993±0.004	0.999±0.000	0.000±0.000
2014	Wood	66	-0.203±0.051	0.166±0.013	623.123±29.732
	Nelder	66	0.499±0.059	-2.511±0.449	1032.079±136.314
	Morant and Gnanastky	66	0.896±0.020	0.983±0.004	-0.666±0.608
	Cobby and Le Du	66	0.299±0.055	-18.576±4.154	1674.951±265.660
	LEG2	66	0.819±0.023	0.966±0.005	0.000±0.000
	LEG3	66	0.908±0.020	0.985±0.003	0.000±0.000
	LEG4	66	0.969±0.012	0.998±0.001	0.000±0.000
2015	Wood	88	-0.424±0.048	0.089±0.011	816.425±31.741
	Nelder	88	0.454±0.047	-1.100±0.249	934.978±120.506
	Morant and Gnanastky	88	0.914±0.011	0.985±0.002	1.207±1.462
	Cobby and Le Du	88	0.117±0.048	-32.816±3.742	3805.829±269.271
	LEG2	88	0.84±0.0170	0.975±0.003	0.000±0.000
	LEG3	88	0.910±0.012	0.986±0.002	0.000±0.000
	LEG4	88	0.958±0.007	0.993±0.001	0.000±0.000

The LEG4 model performed better than the other models in its characterization of the lactation curves of Awassi sheep. The comparison criteria for the model selection included the coefficient of correlation (r) between the observed and estimated lactation curves, the average error (AE) and the coefficient of determination (R^2). The models of Cobby and Le Du, Nelder and Wood had the lowest performance in terms of AE, while the remaining models had AE values that were indistinguishable from zero. The values obtained from the other models were smaller. Taking HO, R^2 and r parameters together, LEG4 was found to best characterize the lactation curves of the Awassi sheep used in the study. In light of these findings, LEG4 was selected as the evaluation model for the study for the identification of features of the lactation curve, as well as the environmental factors affecting the lactation curve and OLMY. Table 4 presents the least square means of the OLMY, LD and model parameters.

The present study also compares the conventional models widely used in lactation curve studies with more recent models. Milk controls were carried out every 20 days to reduce error and to allow a more accurate calculation of OLMY, and the observed and estimated milk yields were found to be very close to one another. Lambings started in different months, as in 2013, when the project began, the flock was brought together from different farmers. Despite all of these issues, OLMY and ELMY overlapped to a significant extent. A rapid decline was noted following the peak in the lactation curve of ewes who lambed in 2015, apart from irregularities in peak levels observed for other years as well, a decline that can be attributed to the harsh winter conditions following lambing in 2015, and the replacement of shepherds.

Table 4. Least square means of model parameters and observed (OLMY) and estimated (ELMY) lactation milk yields by years

			a		b-c		d-q		q		OLMY		ELMY	
Year of birth	n	Month	$\bar{X}_{ay} \pm S_{\bar{x}}$	$\bar{X}_{yıl} \pm S_{\bar{x}}$	$\bar{X}_{ay} \pm S_{\bar{x}}$	$\bar{X}_{ay} \pm S_{\bar{x}}$	$\bar{X}_{ay} \pm S_{\bar{x}}$	$\bar{X}_{yıl} \pm S_{\bar{x}}$	$\bar{X}_{ay} \pm S_{\bar{x}}$	$\bar{X}_{yıl} \pm S_{\bar{x}}$	$\bar{X}_{ay} \pm S_{\bar{x}}$	$\bar{X}_{yıl} \pm S_{\bar{x}}$	$\bar{X}_{ay} \pm S_{\bar{x}}$	$\bar{X}_{yıl} \pm S_{\bar{x}}$
2013	3	December	561.11±240.573^{ab}	1034.66±70.73^{A}	155.29±139.235	-136.13±40.39^{A}	63.68±169.565	-18.69±47.89	46.72±124.129	-18.69±47.89	54.95±22.318	99.97±6.67	54.93±22.297	99.97±6.67
	7	January	662.43±164.669^{a}		36.32±95.304		80.39±116.065		129.66±84.965		67.32±15.276		67.37±15.262	
	35	February	1120.59±80.645^{ab}		-180.47±46.674		-44.78±56.842		-65.39±41.611		109.02±7.481		109.05±7.474	
	14	March	1352.94±123.303^{b}		-245.63±71.363		-46.21±86.909		-67.15±63.621		128.67±11.439		128.68±11.428	
2014	7	January	920.05±152.907	1126.81±62.51^{AB}	-397.72±88.497	-303.82±35.69^{B}	-171.00±107.775	-49.90±42.32	-73.35±78.896	-49.90±42.32	86.95±14.185	107.20±5.90	86.94±14.172	107.18±5.90
	49	February	1249.63±68.269		-304.14±39.512		-19.40±48.119		10.34±35.225		118.69±6.333		118.69±6.327	
	10	March	1420.73±140.962		-340.62±81.583		-37.63±99.355		35.98±72.732		140.25±13.077		140.21±13.065	
2015	14	November	675.52±121.867^{a}	1239.37±68.61^{B}	-8.71±70.532^{ab}	-142.32±39.17^{A}	69.24±85.897^{ab}	41.40±46.45	-15.01±62.880^{ab}	41.40±46.45	54.50±11.306^{a}	113.52±6.47	54.41±11.295^{a}	113.62±6.47
	61	December	1189.22±81.481^{b}		-211.88±47.158^{a}		-56.60±57.431^{a}		-92.04±42.042^{a}		108.54±7.559^{b}		108.74±7.552^{b}	
	13	January	1429.56±117.341^{b}		48.62±67.913^{b}		294.03±82.707^{b}		218.07±60.545^{b}		136.79±10.886^{b}		136.83±10.875^{b}	

Description of Model Parameters

Parameters representing changes in the lactation curve.

- Parameter *a*: the estimated daily milk yield in the middle of lactation, the milk yield when corrected time is zero.
- Parameters b – c: the ups and downs in milk yield in the first half of the lactation period.
- Parameters d – q: the ups and downs in the second half of the lactation period (Silvestre *et al*., 2006; Koncagül *et al*., 2012).

Effects of Environmental Factors

The environmental factors that affect the lactation parameters, year and month of birth, type of birth, and OLMY and ELMY effects, were examined. The effects of birth year and month on parameter ***a*** (milk yield in the middle of the lactation period) varied by year, with parameter ***a*** being observed to increase significantly (P<0.05) with births taking place in later months and over the years. In terms of the effects on parameters ***b-c***, which represent the fluctuations in milk yield from the beginning to the middle of the lactation period, and parameters ***d-q***, representing the fluctuations in the second half of the lactation period, the year of birth was found to have a significant effect on parameters ***b-c*** only (P<0.05). In general, there was an increase in OLMY and ELMY in later birth months and years, although the only significant differences were those between the all months of 2015 (P<0.05). The ewes who started lactating in later months were observed to have higher OLMY values than those who started in earlier months. This can be attributed to conditions in the Şanlıurfa province, where, despite some year-to-year fluctuations, the pasture conditions improve with spring, meaning that ewes that started lactating close to the beginning of spring were exposed to better pasture conditions between birth and peak yield.

Relationships Between Parameters

Table 5 reports the correlations between model parameters. In terms of model parameters the highest correlation (r=0.843) was observed between d and q, followed by those between c-d and b-q. The relationship between OLMY and ELMY was found to be very strong (r=0.999). Parameter *a* was also found to have a very strong relationship with OLMY and ELMY. A breeding program based on parameter ***a*** would likely improve the trait of OLMY.

The observed and estimated overall lactation curves similar classical lactation curve and display a regular increase and decrease (Figure1). The observed and estimated lactation milk yields overlapped to a significant extent.

Table 5. Coefficients of correlation between model parameters of the LEG4 function, OLMY, ELMY and LD.

	b	c	d	q	LSV	TLSV	LS
a	0.026	-0.248**	0.019	-0.026	0.911**	0.911**	0.300**
b		0.450**	0.531**	0.619**	-0.055	-0.055	0.105
c			0.769**	0.600**	-0.332**	-0.332**	-0.215**
d				0.843**	-0.074	-0.074	0.007
q					-0.116	-0.116	-0.066
LSV						0.999**	0.606**
TLSV							0.607**

*P<0.05, **P<0.01, a, b, c, d, q: Fourth order Legendre polynomial model parameters, OLMY: Observed lactation milk yield, ELMY: Estimated lactation milk yield based on model parameters, LD: Lactation length.

Figure 1. Observed and estimated lactation curve.

Discussion

The lactation curves of ewes have been studied by many researchers, but have usually been based on only partial lactation data, as the general practice in sheep husbandry is to focus on milk yield records after the weaning of lambs. This corresponds to a period after peak yield, which makes it impossible to estimate the shape of the curve from the beginning of lactation up until peak yield (Pollott and Gootwine, 2000). Lactation following peak yield usually follows a linear trajectory, which can be characterized by simple linear models. The total lactation milk yields in the present study were observed to be similar to those reported by previous studies of Awassi sheep (Seker *et al*., 2000; Dağ *et al*., 2005; Tekel *et al*., 2007). On the other hand, OLMY and ELMY values were found to be lower than those reported by Gootwine and Pollott (2000). Moreover, the obtained lactation curves were found to be consistent with the lactation curve given by Kaymakçı (2006). OLMY increased in the two months following lambing to reach peak yield, after which it followed a declining trajectory.

In the present study, LEG4 was found to be the model that best characterized the lactation curves of Awassi sheep kept under Institute conditions in Şanlıurfa. Ruiz *et al*. (2000) argue that a high positive correlation among the model parameters used to characterize lactation curves would prevent the accurate characterization of the lactation curve. However, the observed lactation curves and the lactation curves estimated using the LEG4 model in the present study overlapped to a significant extent, despite the strong and positive correlation among the model parameters, indicating that Ruiz *et al*.'s (2006) argument does not always apply. Moreover, the highest correlations between OLMY and ELMY in years 2013, 2014, and 2015 were 0.993, 0.969, and 0.958, respectively, indicating that the LEG4 model used in the present study was successful in characterizing the lactation curves.

Birth month was found to have a significant effect (P<0.05) on OLMY in 2015, and this finding is consistent with other studies examining the effects of environmental factors on lactation milk yields in Awassi sheep (Ozbey and Akcan, 2000; Reiad *et al*., 2010). Total milk yield is closely related to the genotype of sheep and the environmental conditions under which they are kept, in addition to other quantitative factors. The fit of lactation curve models used by different researchers naturally varies depending on the species and breed of the sheep. Elvira *et al*. (2013) report that the lactation curves of Lacuna sheep are best characterized by the Pollat Additive and Fractional models, while Ünal *et al*. (2007) found the Wilmink, Wood and Dhanoa models to be more successful in characterizing the lactation curves of Akkaraman sheep, as well as Kıvırcık-Akkaraman and Sakız-Akkaraman crossbreds. Regarding the characterization of the lactation curves of Awassi sheep, on the other hand, Dağ *et al*. (2005) recommend the Cubic model, whereas Yıldız (1997) reported Wood to be the best model, with the Cobby and LeDu, Dhanoa and Wilming models performing equally well. In a study comparing three different lactation models, Çoban and Kayaalp (2013) reported similar results. In a study comparing linear and non-linear models, Pollott and Gootwine (2000) reported similar findings. Findings are similar to those reported by Esenbuğa and Bilgin (2004) in their study on the lactation curves of Awassi sheep. The model identified as the best performer in characterizing the lactation curve can be used to identify ewes with low yields and remove them from the flock. This would have a direct effect on profitability and efficiency. The LEG4 model used in the present study was found to perform better than the other models. The ELMY estimated using the LEG4 model was found to be very close (0.958<r<0.993) to the OLMY calculated using the Flesichmann method. The LEG4 model can be used to predict the lactation milk yields of Awassi sheep kept under conditions similar to those prevailing in the Şanlıurfa province of Turkey.

Conclusion

Despite the widespread practice in Şanlıurfa of using November and December as the lambing period, it would be better, in terms of total lactation yield, to have the lambings in January and February. It was also found that twin births were no different in terms of milk yield when compared to single births, and farmers in the region usually prefer single births.

The milk yields of Awassi sheep were found, as expected, to be high, and the findings of the present study suggest that it is possible to improve yields of even further through breeding. The findings of the present study can be expected to provide guidance in future breeding efforts with the goal of increasing milk yields. When the HO, R^2 and r parameters were considered together, the fourth-order Legendre polynomial (LEG4) model was found to best predict the lactation curves of Awassi sheep. This study is expected to make a significant contribution to the literature through its examination of regression models in detail, to apply these models to the Awassi dairy species and interpret the results, and to provide future researchers with valuable information.

Acknowledgements

This study is a summary of the first author's PhD thesis. The data were obtained from Şanlıurfa GAP Agricultural Research Institute of the General Directorate of Agricultural Research and Policies of the Ministry of Agriculture and Forestry making use. Project No: TA-

GEM/11/08/01/01. Restrictions apply to the availability of these data, which were used with permission for this study. The authors are thankful to, The General Directorate of Agricultural Research and Policies (GDARP) and Şanlıurfa GAP Agricultural Research Institute, to provide the necessary animal material to carry out the research program.

References

Beever, D. E., Rook, A. J., France, J., Dhanoa, M. S., & Gill, M. (1991). A review of empirical and mechanistic model of lactation performance by the dairy cow. Livestock Production Science, 29, 115-130. https://doi.org/10.1016/0301-6226(91)90061-T

Cobby, J. M., & Le Du, Y. L. P. (1978). On fitting curves to lactation data. Animal Science, 26(2), 127-133. https://doi.org/10.1017/S0003356100039532

Çoban, F., & Kayaalp, G. T. (2013). Süt Koyunculuğunda Laktasyon Eğrisi Modellerinin Karşılaştırmalı Olarak İncelenmesi [Comparative Analysis of Lactation Curve Models in Dairy Sheep Farming]. Çukurova Üniversitesi Fen ve Mühendislik Bilimleri Dergisi, 29(1), 80-88.

Dağ, B., Keskin, I., & Mikailsoy, F. (2005). Application of different model to the lactation curves of unimproved Awassi Ewes in Turkey. The South African Journal of Animal Science, 35(4), 238-243.

Elvira, L., Hernandez, F., Cuesta, P., Cano, S., Gonzalez-Martin, J., & Astiz, S. (2013). Factors affecting the lactation curves of intensively managed sheep based on a clustering approach. Journal of Dairy Research, 80(4), 439-447. https://doi.org/10.1017/S0022029913000381

Epstein, H. (1982). Awassi Sheep, World Animal Review, Editor, D.E. Faulkner. FAO.

Esenbuğa, N., & Bilgin, Ö. C. (2004). İvesi koyunlarının laktasyon eğrisinin tahmini ve tanımlanması için farklı matematik modellerin mukayesesi [Comparison of different mathematical models for estimation and definition of the lactation curve of Ivesi sheep]. Ulusal Zootekni Bilim Kongresi, 166 – 169. 1-3 Eylül 2004. Süleyman Demirel Üniversitesi.

Gipson, T. A., & Grossman, M. (1990). Lactation curves in dairy goats: A review. Small Ruminant Research, 3, 383-396.

Gootwine, E., & Pollott, G. E. (2000). Factor saffecting milk production in Improved Awassi dairy ewes. Animal Science, 71, 607-615.

Grossman, M., Kuck, A. L., & Norton, H. W. (1986). Lactation Curves of Pure bred and Cross bred Dairy Cattle. Journal of Dairy Science, 69, 195-203.

Güngör, I., & Akçapınar, H. (2013). The Production Traits of Bafra Sheep Reared in Ankara Conditions. Livestock Studies, 53-2, 059-073.

Keskin, I., & Dağ, B. (2000). Comparison of different mathematical models for describing the complete lactation of akkaraman ewes in Turkey. Asian-Australasian Journal of Animal Sciences, 19, 1551–1555.

Kirkpatrick, M., Lofsvold, D., & Bulmer, M. (1990). Analysis of the inheritance, selection and evolution of grow thtrajectories. Genetics, 124, 979–93.

Koncagül, S., Daşkıran, I., & Bingöl, M. (2012). Yetiştirici Elinde Bulunan Norduz Koyunlarının Laktasyon Süt Verimi ve Laktasyon Eğrisine Etki Eden Faktörler [Lactation Milk Yield and Factors Affecting the Lactation Curve of Norduz Sheep Breeders]. Kafkas Universitesi Veteriner Fakültesi Dergisi, 18(4), 677-684.

Morant, S. V., & Gnanasakthy, A. (1989). A new approach to the mathematical formulation of lactation curves. Animal Science, 49, 151-162.

Nelder, J. A. (1966). Inverse Polynomials. A Useful Group of Multi-factor Response Functions. Bio-metrics, 22, 128-144.

Ozbey, O., & Akcan, A. (2000). Production performance of Akkaraman, Morkaman and Awasi sheep under semi-intensive conditions I. Fertility and milk production characteristics. Veteriner Bilimleri Dergisi, 16(1), 109-120.

Pollott, G. E., & Gootwine, E. (2000). Appropriate mathematical models for describing the complete lactation of dairy sheep. Animal Science, 71, 197–207.

Prakash, V., Gupta, A. K., Gupta, A., & Gandhi, R. S. (2016). Lactation curve modeling using Legendre Polynomial in Sahiwal cattle. The Indian Journal of Animal Sciences, 86(4), 485–488.

Reiad, K., Al-Azzawi, W., Al-Najjar, K., Masri, Y., Salhab, S., Abdo, Z., El-Herek, I., Omed, H., & Saatci, M. (2010). Factor influencing the milk production of Awassi sheep in a flock with theve selected lines at the Agricultural Scientific Research Center in Salamieh-Syria. Kafkas Universitesi Veteriner Fakultesi Dergisi, 16(3), 425-430.

Ruiz, R., Oregui, L. M., & Herrerot, M. (2000). Comparison of models for describing the lactation curve of latxa sheep and an analysis of factors affecting milk yield. Journal of Dairy Science, 83, 2709–2719.

Sakul, H., & Boylan, W. J. (1992). Lactation curves for several US sheep breeds. Animal Production Science, 54, 229-233.

SAS. (2000). SAS/STAT. SAS Inst Inc, Cary, NC, USA.

Schaeffer, L. R. (2004). Application of random regression models in animal breeding. Livestock Production Science, 86, 35-45.

Seker, I., Kul, S., & Bayraktar, M. (2000). Linear udder traits and relationships between these and milk yield in Awassi and East-Friesian x Awassi crossbreed (F_1) ewes. Lalahan Hayvancılık Araştırma Enstitüsü Dergisi, 40(2), 45-55.

Silvestre, A. M., Petim-Batista, F., & Colaço, J. (2006). The accuracy of seven mathematical functions in modeling dairy cattle lactation curves based on test-day records from varying sample schemes. Journal of Dairy Science, 89, 1813-1821.

TAGEM. (2009). Türkiye Evcil Hayvan Genetik Kaynakları Kataloğu [Turkey Domestic Animal Genetic Resources Catalog]. T.C. Tarım ve Köyişleri Bakanlığı, Tarımsal Araştırmalar Genel Müdürlüğü, 34-35.

Tekel, N., Sireli, H. D., & Elicin, M. A. (2007). Research on the repeatability of milk yield in Awassi sheep. Harran Üniversitesi Ziraat Fakültesi Dergisi, 11(1/2)49-53.

Tekerli, M., Akinci, Z., Dogan, I., & Akcan, A. (2000). Factors Affecting the Shape of Lactation Curves of Holstein Cows from the Balikesir Province of Turkey. Journal of Dairy Science, 83, 1381–1386.

TUIK. (2019). Animal Production Statistics. Ankara, Turkey. TUIK.

Tzouramani, I., Sıntorı, A., Lıontakıs, A., Karanikolas, P., & Alexopoulos, G. (2011). An assessment of the economic performance of organic dairy sheep farming in Greece. Livestock Science, 141, 136–142.

Ünal, N., Orman, M. N., Çolak, C., Atasoy, F., Mundan, D., & Aytac, M. (2000). Comparision of various models for lactation curves of Akkaraman and its crossbreds (Chios x Akkaraman F1 and Kıvırcık x Akkaraman F1) ewes. Lalahan Hayvancılık Araştırma Enstitüsü Dergisi, 47(2), 7-13.

Wood, P. D. P. (1967). Algebraic model of the lactation curve in cattle. Nature, 216, 164-165

Yıldız, A. (1997). Lactation curve of Awassi sheep and affecting factors in the State of Ceylanpınar Farm. Ph.D. Thesis, Fırat Üniversitesi Sağlık Bilimleri Enstüsü Elazığ.

Yılmaz, O., Denk, H., & Nursoy, H. (2004). Milk yield characteristics of Norduz sheep. Van Veterinary Journal, 15(1-2), 27-31.

Livestock Studies, 2020; 60 (2): 54-61
http://doi.org/10.46897/livestockstudies.846415

RESEARCH PAPER

LIVESTOCK STUDIES

The effect of some environmental factors on lactation length, milk yield and calving intervals of Anatolian Buffaloes in Bartın province of Turkey

Kürşat Alkoyak[1*], Sezer Öz[1]

[1] Republic of Turkey Ministry of Agriculture and Forestry, General Directorate of Agricultural Research and Policies, Department of Livestock and Aquaculture Research Ankara, Turkey.

Article History

Received: 09 September 2020
Accepted: 10 December 2020
First Online: 25 December 2020

Corresponding Author

Tel.:+ 903123157623 -1255
E-mail:kursatalkoyak@gmail.com

Keywords

Anatolian buffalo
Environmental factors
Lactation length
Milk yield
Calving interval

Abstract

This study aims to determine the environmental factors effected to lactation length (LL), lactation milk yield (LMY), calving intervals (CI) of Anatolian buffaloes in Bartın. In this study, 1511 milk yield data belonging to 762 Anatolian buffaloes and 957 CI data belonging to 543 Anatolian buffaloes which has been reared in different environmental conditions between 2015-2019 under the scope of the Anatolian Buffalo Breeding Project being conducted in Bartın province. The least squares method was used for determining the effect of environmental factors, and Tukey multiple comparison tests were used for multiple comparison. Mean and standard deviations relevant to the LL, LMY, and CI were detected as 260.26 ± 1.33 days, 1035.5 ± 8.21 kg, and 426.35 ± 2.91 days, respectively. County, calving year, and season, age, and lactation number's effects on those parameters were investigated. The effect of calving year (P<0.001) county and season (P<0.01) on LL; county, calving year (P<0.001) and calving age (P<0.05) on LMY; lactation number (P<0.001) and calving age (P<0.01) and season (P<0.05) on CI were found statistically significant. Meanwhile, a highly significant positive phenotypic correlation was calculated between LMY and LL (r = 0.66, P <0.001). There are no adequate studies related to the environmental factors influencing lactation length, lactation milk yield, calving intervals of Anatolian buffaloes. Significant environmental factors detected in this study should be considered in selection programs.

Introduction

Buffalo (*Bubalus Bubalis*) is an extensively reared as a dairy animal and originated from Asia (Borghese, 2010). While the world's buffalo population was 173 million in 2005, it has reached 206.6 million in 2018. The increasing percentage in the world's buffalo population between 2005-2018 is 19.4 (FAO, 2020). Buffaloes have great importance in regards to the amount of milk produced and milk's nutritional composition. Total buffalo milk production in 2018 was 127.7 million tonnes in the world. This amount constitutes around 15% of total milk production of the world, therefore buffaloes have become the second highest milk producers of the world after dairy cattle (FAO, 2020).

Buffaloes have been reared in Turkey originated from Mediterranean Buffaloes, which are the sub-group of Riverin Buffaloes and named as Anatolian Buffaloes (Cicek et al., 2009). Buffaloes mainly reared at the Northern, Middle, Marmara, Eastern, and South-Eastern Regions of Turkey (Atasever and Erdem, 2008). While Turkey's buffalo population was 105 thousand in 2005, it has reached 184 thousand in 2019 thanks to the National Anatolian Buffalo Breeding Project in Farm Conditions implemented by the Turkish Ministry of Agriculture and Forestry. There are 1.659 buffaloes reared currently in Bartın province located in the Northwest part of Turkey (TUIK, 2020a). Buffaloes being bred in Turkey for milk and meat production. In addition to their resistance against

diseases and harsh environmental conditions, not only the higher capability of feed efficiency, converting poor-quality feedstuffs into high-quality milk and meat, but also lower expenses needed for husbandry compared to dairy cattle could be considered as other main reasons for engaging in buffalo husbandry (Atasever and Erdem, 2008). Buffalo milk is used in order to produce yoghurt, cream, cheese, and ice cream, and it contains 7.92% fat and 4.09% protein. Meanwhile, buffalo meat is used for producing salami (Soysal et al., 2015).

According to TUIK (2020b) 79 thousands tonnes of milk and 73 tonnes of meat produced from buffaloes in 2019 in Turkey. To increase the efficiency of dairy buffaloes, factors that influence milk production should be ameliorated. Total milk production of buffaloes; effected by non-genetic parameters such as season, management, and feed quality (Afzal et al., 2007; Pawar et al., 2012) some other parameters like calving interval and age are closely related to efficient milk production (Khosroshahi et al., 2011). LMY and LL are significant parameters for dairy buffaloes (Chaudhry, 1992).

There is not adequate research about the environmental factors which influence LL, LMY, and CI of Anatolian Buffaloes. Hence, more studies required to determine the effects of environmental parameters on production of Anatolian Buffaloes. This study aims to examine siginificant environmental factors influencing LL, LMY, and CI of Anatolian buffaloes.

Materials and Methods

Animal material of the study comprises of pedigree records of 762 Anatolian buffaloes' milk yield records and 543 calving interval records in Bartın province (410 38' 28'' N and 320 19' 59'' E) between 2015-2019. In this study, 1511 milk yield records and 957 calving interval records were evaluated and obtained from Anatolian buffaloes that were born between these years. LMY and CI records procured from the database named 'Manda Yıldızı'. The data being uploaded by project technical staff hired for The National Anatolian Buffalo Breeding Project which has been coordinated and supported by the General Directorate of Agricultural Research and Policies (Tekerli, 2019).

Buffaloes are milked twice daily, at morning and evening. Milk records are collected each month with a precision scale which is sensitive to 10 g / 50 kg. Anatolian buffaloes with at least the 4 months of lactation data were included in the analysis (Koç and Kızılkaya, 2009). The records between 123≤LL≤404 d for LL and 300≤CI≤700 d for CI (Soysal et al., 2018) were evaluated in this study. The data of animals leaving the herd before the lactation ended due to diseases, sales, and deaths were not evaluated. Abort and stillbirths were excluded in the calculation of the CI.

Buffalo husbandry is carried out under extensive conditions plus similar management and nutritional conditions in the region where the research was conducted. Farmers do not usually apply additional feeding to buffalos particularly in the pasture period, but they do additional feeding according to the current feed (straw, alfalfa, silage, etc.) in winter. On the days when seasonal conditions are suitable for grazing in the region, buffaloes are taken to pasture after morning milking. While most of the farmers are milking by hand, fewer are milking with a machine. This study was conducted in 4 counties; (1) Amasra, (2) Kurucaşile, (3) Center and (4) Ulus. Calving year was grouped between 2015 to 2019. Based on the geo-climatic conditions prevailing in Turkey, calving seasons allocated into four groups; (1) winter (December, January and February), (2) spring (March, April and May), (3) summer (June, July and August) and (4) autumn (September, October and November). Age was divided into five groups: (1) 3-4 yrs, (2) 5-6 yrs, (3) 7-8 yrs, (4) 9-10 yrs, (5) 11 yrs and older. Lactation number was listed numerically one through five.

Among the environmental factors examined in this study, the effect share of the county, calving year, season, age, and lactation number on LL, LMY, CI and were determined using the least squares method. The test of significance in terms of statistics were made by variance analysis and the differences between the averages were evaluated by the Tukey multiple comparison tests. Besides, a phenotypic correlation was calculated from unadjusted data via Pearson Method. The GLM (General Linear Model) procedure in the "Minitab-Version 18" program package was used for statistical analysis of the data (Minitab, 2017).

The effects of various environmental factors on lactation length, lactation milk yield, and calving interval were examined using the model below.

$Y_{ijklmn} = \mu + C_i + Y_j + S_k + A_l + L_m + e_{ijklmn}$ as follows;

Y_{ijklmn}: The yield characteristics of any buffalo (i: county, j: year, k: season, l: age, m: Lactation number)

μ: Overall (expected) average,

$C_{i:}$ The effect of County (i= 1,2,3,4),

Y_j: The effect of Calving Year (j= 2015, 2016, 2017, 2018, 2019),

S_k: The effect of Calving Season (k= 1,2,3,4),

A_l: The effect of Calving Age (l=1,2,3,4,5)

L_m: The effect of Lactation Number (m= 1, 2, 3, 4, 5),

e_{ijklmn}: Random error which is assumed to be normally independently distributed with zero mean and constant variance (NID, 0, σ2).

Results

In this study, LL, LMY, CI overall mean and standard error were determined as 260.26 ± 1.33 days, 1035.5 ± 8.21 kg, and 426.35 ± 2.91 days, respectively (Table 1). The effects of some environmental factors on LL, LMY, and CI in Anatolian buffaloes are examined and the mean, standard errors, and effective factors of these characteristics are given in Table 2.

Table 1. Descriptive statistics for LL, LMY, and CI in Anatolian buffaloes

Number of Animals	Number of Records	Mean	Standard Error (S)	Minimum	Maximum
Lactation Length (days)					
762	1511	260.26	1.33	123	404
Lactation Milk Yield (kg)					
762	1511	1035.5	8.21	294.9	1986.6
Calving Interval (days)					
543	957	426.35	2.91	300	700

Table 2. Least squares means (± SE) of lactation number, LMY, and CI according to the county, calving year, season, and age, lactation number of Anatolian buffaloes

Factors		Lactation Length (days)		Lactation Milk Yield (kg)		Calving Interval (days)	
		n	(Mean±SE)	n	(Mean±SE)	n	(Mean±SE)
County	P		**		***		NS
	Amasra	73	278.34±6.05^{a}	73	1145.0±32.0^{a}	55	399.0±12.5
	Kurucaşile	65	246.97±6.52^{b}	65	1045.4±34.5^{ab}	33	397.9±15.9
	Center	1201	257.66±2.11^{b}	1201	1026.4±11.2^{b}	767	402.45±5.34
	Ulus	172	255.72±4.16^{b}	172	1100.3±22.0^{a}	102	395.39±9.46
Calving Year	P		***		***		***
	2015	236	263.66±4.59^{ab}	236	870.6±24.3^{d}	98	363.10±12.2^{d}
	2016	309	258.40±4.04^{b}	309	973.4±21.4^{c}	195	383.45±9.41^{cd}
	2017	425	268.26±3.61^{ab}	425	1182.8±19.1^{b}	263	397.30±8.25^{bc}
	2018	431	272.21±3.23^{a}	431	1251.2±17.1^{a}	326	425.91±6.95^{a}
	2019	110	235.82±5.23^{c}	110	1118.4±27.7^{b}	75	423.50±11.1^{ab}
Calving Season	P		**		NS		*
	Winter	221	265.35±4.09^{a}	221	1113.4±21.6	137	418.04±9.34^{a}
	Spring	398	261.61±3.59^{a}	398	1072.7±19.0	230	390.28±8.57^{b}
	Summer	523	251.68±3.29^{b}	523	1058.7±17.4	333	390.62±7.50^{b}
	Autumn	369	260.05±3.69^{ab}	369	1072.4±19.6	257	395.71±8.32^{ab}
Calving Age (year)	P		NS		*		**
	3-4	348	259.88±5.19	348	1020.3±27.5^{b}	51	357.0±15.1^{c}
	5-6	531	256.13±3.90	531	1054.8±20.6^{b}	333	388.36±8.71^{bc}
	7-8	338	258.47±3.79	338	1072.6±20.0^{ab}	298	405.52±7.69^{ab}
	9-10	200	268.09±4.47	200	1140.9±23.6^{a}	183	408.72±8.35^{ab}
	11≤	94	255.79±6.46	94	1107.8±34.2^{ab}	92	433.70±11.5^{a}
Lactation Number	P		NS		NS		***
	1st	470	258.21±4.54	470	1022.1±24.0	374	452.97±7.84^{a}
	2nd	420	259.93±3.94	420	1068.2±20.9	322	422.79±7.91^{b}
	3rd	352	258.73±3.93	352	1092.3±20.8	204	381.97±9.39^{c}
	4th	211	260.76±4.76	211	1103.4±25.2	57	336.90±13.9^{d}
	5th	58	260.72±7.54	58	1110.5±39.9	-	-

* : P<0.05 ** : P<0.01 *** : P<0.001 NS : Non-Significant (P>0.05)
a, b, c, d : Means in a column with different superscripts differ significantly (P<0.05).

The effects of the county, calving year, season, calving age, and lactation number on these characteristics were determined. The effect of calving year (P <0.001) county and season (P <0.01) on LL; the effect of calving age (P <0.05) on LMY was found important, and the effect of calving year and the county on calving age was considered significantly important (P <0.001). While the effects of calving season on CI (P <0.05) and calving age (P <0.01) were found statistically significant, the effects of calving year and lactation number on CI were found to be significant (P <0.001). The high positive phenotypic correlation was calculated between LMY and LL (r = 0.66, P <0.001). In addition, low positive phenotypic correlations were calculated between CI and LL (r = 0.15, P <0.001) and between CI and LMY (r = 0.13, P <0.001) (Table 3).

Table 3. Phenotypic correlation (rP) for some traits of Anatolian buffaloes

Traits	Lactation Length	Lactation Milk Yield	Calving Interval
Lactation Length	-		
Lactation Milk Yield	0,66***	-	
Calving Interval	0,15***	0,13***	-

*** : P<0.001

Discussion

The LL obtained (260.26 ± 1.33 days) (Table 1) in this study is longer than the lengths found in Şahin and Ulutaş (2014) (146.6 days), Tekerli *et al.* (2016) (229.4 days), Uğurlu *et al.* (2016) (231.9 days), and Koçak *et al.* (2019) (245.4 days) studies on Anatolian buffaloes. The reason for this case could be taken into account under the following conditions, farmers would like to obtain maximum milk from buffaloes as long as possible, ignoring the economy of lifelong milk production (Hussain *et al.*, 2006). The LL found in the study was relatively short but similar to Rosati and Van Vleck (2002) (270 days) findings in Italian buffaloes, Afzal *et al.* (2007) (273.3 days) in Nili Ravi buffaloes in Pakistan. However, the LL obtained at the end of this study is shorter than the values reported by Cady *et al.* (1983) (282 days) and Chaudhry (1992) (302 days) as a result of some researches on Nili Ravi buffaloes. The differences in LL values might be due to various management and feeding programs implemented in the farms.

The effect of county on LL was found significant (P <0.01) in this study in accordance with Soysal *et al.* (2018) findings carried out a study on Anatolian buffaloes reared in İstanbul. The highest LL was calculated in Amasra, the lowest was in Kurucaşile. The effect of calving year on LL was determined significant (P <0.001) in this study (Table 2) compatible with the studies carried out by Charlini and Sinniah (2015) from Sri Lanka and Koçak *et al.*(2019) on Anatolian buffaloes. In this study, the longest LL was obtained in 2018, while the shortest LL was achieved in 2019. Alterations in LL during various calving years may be due to climate factors and/or differences in management practices at the farm. The effect of calving season on LL was found significant (P <0.01) in this study (Table 2) similar to the studies conducted by Hussain *et al.* (2006) on Nili Ravi buffaloes, Şahin and Ulutaş (2014), and Koçak *et al.* (2019) on Anatolian buffaloes. Besides, Bashir *et al.* (2015) also reported that the LL was affected by the calving season. Nevertheless, the abovementioned effect was found non-significant in some studies (Ghaffar et al., 1991; Chaudhry , 1992; Khan and Chaudhry, 2000; Afzal et al., 2007) conducted on buffaloes, in contrast with the findings obtained in this study.

It was observed that Anatolian buffaloes calved in winter have longer LL than calved in summer and autumn as compared with spring (Table 2). Furthermore, the longest LL was achieved in buffaloes calved in winter, while the shortest LL was achieved in buffaloes calved in summer. Similar results were reported by Şahin and Ulutaş (2014) in a study conducted on Anatolian buffaloes in Tokat province of Turkey, contrary to the Khalil *et al.* (1992) 's findings are relevant to the longest LL in buffaloes calved in spring as a result of a study on Egyptian buffaloes. While some researchers reported that the effect of year, season, and age on LL is significant (Hussain et al., 2006; Marai et al., 2009; Koçak et al., 2019), in this study only the effect of age was found non-significant (P>0.05) apart from other factors. Moreover, Soysal *et al.* (2018) reported similar results that the effect of age on LL was not considerable as a result of various studies on Anatolian buffaloes, unlike Khan and Chaudhry (2000)'s reports with respect to the significant effect of calving age on LL.

As a result of this study, no differences were observed (P>0.05) in Anatolian buffaloes in terms of the effect of lactation number on LL. Similar results were obtained by Afzal *et al.* (2007) in consequence of a study carried out Nili-Ravi buffaloes in Pakistan. However, the effect of lactation number on LL was found significant in most studies conducted on buffaloes. These differences

might be shown up due to various farm management conditions and various locations of farms. As can be understood from this research (Table 2), as lactation number increases, LL values increase properly even though they are not very dissimilar. Nevertheless, Cady *et al.* (1983) reported that as lactation number increases, LL values reduce.

The LMY (1035.5±8.21 kg) (Table 1) obtained in this study is higher than Tekerli *et al.* (2001) (894.3 kg), Şahin and Ulutaş (2014) (657.7-761.4 kg) and Uğurlu *et al.* (2016) (925.4 kg) reported. On the other hand, it is similar to the value reported as 1000.7 kg in the study conducted by Tekerli *et al.* (2016) on different originated Anatolian buffaloes. However, this value found in the study is less than the LMY reported by many other researchers. According to some researches carried out on various buffalo breeds from various countries, LMY reported as; Cady *et al.* (1983) (1883 kg), Khan and Chaudhry (2000) (1984 kg) at Nili Ravi buffaloes reared in Pakistan and Rosati and Van Vleck (2002) reported 2286 kg at Mediterranean buffaloes reared in Italy. These production levels are considerably higher than the milk yield of Anatolian Buffaloes reared in Turkey. Likewise, the milk yield value found in this study is lower than the value obtained by Şekerden (2011) (1300 L) as a result of the study carried out on Anatolian buffaloes in Turkey. These differences in milk yield may result from differences in nutritional and management practices (Charlini and Sinniah, 2015).

The effect of county on LMY was found significant (P <0.001) in this study. Soysal *et al.* (2018) was found the effect of region significant in their study on Anatolian buffaloes in İstanbul, which is consistent with this study. The highest LMY was calculated in Amasra, while the lowest in Central County. These results suggest that Amasra is a suitable region for buffalo breeding. The effect of the calving season on LMY found non-significant (P>0.05) in this study. It was observed that buffaloes that calved during winter and autumn seasons possess more milk production than calved at the other seasons (Table 2). Soysal *et al.* (2018) found the effect of season on LMY was important contrary to these findings. However, they reported the highest milk yield from buffaloes calved during the autumn and winter seasons as well. Similarly, Catillo *et al.* (2002) have determined the highest LMY at buffaloes calved in winter and lowest in summer. Şahin and Ulutaş (2014) stated that the main reasons for lower milk yields in summer are temperature stress, the vegetation of pastures, and difficulties at feed supply. On the other hand, the low LL of buffaloes that calved in summer in this study may have caused LMY to be lower than other seasons.

Buffaloes are influenced by different air temperatures in different seasons. They stay longer in barn in the winter, therefore milking for a longer time by feeding inside could provide an opportunity to obtain higher milk yield from buffaloes calved during winter. The region where the research is conducted comprises generally family-type traditional farms. Produced buffalo milk is sold in local markets either as raw milk or by converting it into various dairy products (usually buffalo yoghourt) can thus contribute substantially to the family economy. Considering all these factors, a high level of LMY of buffaloes that are reared in intensive conditions during the winter is an indication of given importance to buffaloes in this period in terms of care and nutrition. Similarly to these findings, Kul *et al.* (2016) in Anatolian buffaloes and Ghaffar *et al.* (1991) in Nili-Ravi buffaloes reported that calving season had no significant effect on milk production. Afzal *et al.* (2007) stated that climate stress factors can be minimized and overcome through better nutrition and management.

The effect of calving year (P <0.001) and age (P <0.05) on LMY were found significant in the study. The highest milk yield was reached in 2018, while the lowest milk yield was achieved in 2015 (Table 2). The alteration in milk yield observed in different years reflected the level of management and environmental impacts at the farm. The level of management varies according to the skills of farmers, cultivating and rearing system, selection method, and density (Khan, 1986). Catillo *et al.* (2002), Şahin and Ulutaş (2014) findings confirmed the noticeable effect of calving age on LMY. In this study, the highest LMY determined from buffaloes calved at 9-10 years of age and lowest LMY at 3-4 years of age. It was observed that LMY gradually increased after the age of calved at the age of 9-10 and decreased after the age of 11≤. Similar results obtained by Koçak *et al.* (2019) the highest milk yield at the age of 9, and the lowest reported at ≤4. Bashir *et al.* (2015) emphasized that age may be a more precise factor to be included in models to be utilized in lactation milk yield. Since the culling of buffaloes with lower milk yield from the herd and the expansion of this process contribute to obtaining better LMY than the herd in subsequent lactations (Khan et al., 1997).

The effect of lactation number on LMY was found statistically non-significant (P>0.05), similar to Pawar *et al.* (2012)'s findings. On the contrary, Afzal *et al.* (2007) reported that the effect of lactation number on LMY was found statistically significant. Lowest LMY was obtained at 1th, while the highest was achieved at 4th and 5th. lactations confirming Marai *et al.* (2009)'s findings related to reaching highest LMY at 4th and 5th lactation number. Furthermore, Afzal *et al.* (2007) and Khosroshahi *et al.* (2011)'s findings have promoted these results by their reports for obtaining the lowest LMY at 1. lactation. It

was observed that in this study, as lactation number increased, LMY increased regularly (Table 2). This increased milk production in subsequent lactations can be explained by the continuation and maturation of the mammary gland.

The average CI value for Anatolian buffaloes (426.35 ± 2.91 days) (Table 1) was determined higher than the values found in the studies by Marai *et al.* (2009) (402.6 day) on Egyptian buffaloes and Soysal *et al.* (2018) (417 days) on Anatolian buffaloes. Although CI was relatively high in the study conducted by Tekerli *et al.* (2001) they found as 441.9 days similar to this study. On the other hand, from India conducted by Hussain *et al.* (2006) (473.7 days) on Nili-Ravi buffaloes, Charlini and Sinniah (2015) (470 days) from Sri Lanka, and Koçak *et al.* (2019) (450.3 days) on Anatolian Buffaloes obtained higher CI values than this study.

The effect of county on CI was found non-significant (P >0.05) in this study similar to Soysal *et al.* (2018)'s findings. The highest CI was observed in Central County, while the lowest in Ulus county. This result shows that the buffalo breeders in Ulus county are more meticulous in reproductive traits and estrus monitoring. CI value was linearly decreased, as lactation number increased, coherently with Cady *et al.* (1983) and Charlini and Sinniah (2015)'s findings. The effect of calving season on CI was found statistically significant (P<0.05), similar to the Tekerli *et al.* (2001) and Koçak *et al.* (2019)'s findings at Anatolian buffaloes and Marai *et al.* (2009)'s findings at Egyptian buffaloes. On the other hand, Soysal *et al.* (2018) found the effect of calving season on CI was non-significant. In the study, the longest CI value was observed in Anatolian buffaloes calved in winter, while the shortest value was observed in buffaloes calved in winter. Bashir *et al.* (2015) and Koçak *et al.* (2019) support that results by finding the longest CI value in buffaloes that calved in winter, the shortest CI in buffaloes that calved in summer. The shortest CI obtained from the buffalo calved in summer can be explained by the occurrence of postpartum estrus and conception period in the winter. The reduction at daylight and air temperature in winter and autumn seasons may increase the reproductive activity in the buffaloes. As a matter of fact, Hafez (1955) reported that sexual activity was observed in some buffaloes at the onset of autumn. Likewise, Soysal *et al.* (2018) detected that the effect of calving year, calving age, and lactation number on CI was significant in a study conducted in Anatolian buffaloes. Similar results were reported by some researchers (Hussain et al., 2006; Marai et al., 2009; Charlini and Sinniah, 2015) as the effect of lactation number on CI was significant. On the contrary, Tekerli *et al.* (2001) reported that the effect of lactation number and age on CI was not significant in Anatolian buffaloes. In this study, it was observed that with the increase of calving age, the duration of CI increased. From this, it can be concluded that CI is prolonged as a result of the fact that buffaloes cannot conceive regularly due to reasons such as farm management and abduction of the oestrus cycle.

It was determined that the positive phenotype correlation between LMY and LL is high and siginificant (Table 3). Similar to this study, many researchers reported that the correlations between LMY and LL are high and significant in buffaloes (Khan et al.1997; Afzal et al. 2007; Galsar et al. 2016; Rathod et al. 2018). Phenotypic correlation between CI and LL and between CI and LMY was found significant, positive and very low (Table 3). Rathod *et al.* (2018) and Jakhar *et al.* (2016) reported that the phenotypic correlation between CI and LL is significant and positive, similar to this study. However, unlike this study, the correlation coefficient was found to be high in the same study. Similar to this study, *Rathod et al.* (2018) found a positive and low relationship between CI and LMY. On the other hand, *Jakhar et al.* (2016) reported that there is a significant, low and negative relationship between CI and LMY.

Conclusion

Taking measures to ameliorate maintenance, feeding, and herd management will improve the current situation of the farms and contribute to productivity, in the buffalo farms in Bartın. Among the environmental factors examined in the research; the effects of the county, calving year and age on milk yields, and calving year, season, age, and parity on CI were found to be significant. Stud selection and breeding studies can be done considering these important environmental factors in order to improve milk yield and other performances of buffaloes. Also, calving can be planned according to the winter since milk yield found highest in the buffaloes that calved in the winter.

Acknowledgments

The authors would like to express their most sincere thanks for their contribution to the Directorate General of Agricultural Research and Policies of the Ministry of Agriculture and Forestry and the Provincial Buffalo Breeders Association of Bartın and the Project Technical Staff, which enabled the execution of this study.

References

Afzal, M., Anwar, M., & Mirza, M. A. (2007). Some factors affecting milk yield and lactation length in Nili Ravi buffaloes. Pakistan Veterinary Journal 27(3):113-117.

Atasever, S., & Erdem, H. (2008). Water buffalo raising and its future in Turkey. The Journal of Agricultural Faculty of Ondokuz Mayıs University 23(1):59-64.

Bashir, M. K., Khan, M. S., Lateef, M., Mustafa, M. I., Khalid, M. F., Rehman, S., & Farooq, U. (2015). Environmental factors affecting productive traits and their trends in Nili-Ravi buffaloes. Pakistan Journal of life and Social Sciences 1-8.

Borghese, A. (2010). Development and perspective of Buffalo and Buffalo market in Europe and near east. Revista Veterinaria v.21(sup.), p.20-31.

Cady, R. A., Shah, S. K., Schermerhorn, E. C., & McDowell, R. E. (1983). Factors affecting performance of Nili-Ravi buffaloes in Pakistan. Journal of Dairy Science 66(3): 578-586.

Catillo, G., Macciotta N. P. P., Carretta, A., & Capplo-Borllinot, A. (2002). Effects of age and calving season on lactation curves of milk production traits in Italian water buffaloes. Journal of Dairy Science 85:1298-1306.

Charlini, B. C., & Sinniah, J. (2015). Performance of Murrah, Surti,Nili-Ravi buffaloes and their crosses in the intermediate zone of Sri Lanka. Livestock Research for Rural Development 27(3):47

Chaudhry, M. A. (1992). Factors affecting the lactation length and milk yield in Nili-Ravi buffaloes. Asian-Australasian Journal of Animal Sciences 5(2):375-382.

Cicek, H., Gunlu, A., & Tandogan, M. (2009). Production function analysis of buffalo fattening enterprises in Afyonkarahisar Region of Turkey. Journal of Animal and Veterinary Advances 8(11):2158-2163.

FAO. (2020). Food and Agricultural Organization of the United Nations. (2020) Available in: http://www.fao.org/faostat/en/#data/QA. Accessed on 22 June 2020.

Galsar, N. S., Shah, R. R., Gupta, J. P., Pandey, D. P., Prajapati, K. B., & Patel, J. B. (2016). Genetic estimates of reproduction and production traits in Mehsana buffalo. Indian Journal of Dairy Science. 69(6): 698-701.

Ghaffar, A., Khan, M. I., Mirza, M. A., & Pirzada, W. H. (1991). Effect of year and calving season on some traits of economic importance in Nili-Ravi buffaloes. Pakistan Journal of Agricultural Research 12:217-221.

Hafez, E. S. E. (1955). Puberty in the buffalo-cow. The Journal of Agricultural Science 46:137-142 (Anita. Breed. Abst. 23: 1670).

Hussain, Z., Javed, K., Hussain, S. M. I., & Kiyani, G. S. (2006). Some environmental effects on productive performance of Nili-Ravi buffaloes in Azad Kashmir. Journal of Animal and Plant Sciences 16(3-4):66-69.

Jakhar, V., Vinayak, A. K., & Singh, K. P. (2016). Genetic evaluation of performance attributes in Murrah buffaloes. Haryana Veterinarian 55(1): 66-69.

Khalil, M. H., Afifi, E. A., Bedeir, L. H., & Zeidan, S. M. (1992). Genetic analysis of lactation traits in Egyptian buffaloes. Egyptian Journal of Animal Production 29:155-172.

Khan, M. A. (1986). Genetic analysis of a purebred herd of Nili-Ravi buffaloes. Ph.D Thesis, University of Agriculture, Faisalabad (Pakistan).

Khan, M. S., Shook, G. E., Asghar, A. A., Chaudhary, M. A., & McDowell, R. E. (1997). Genetic parameters of milk yield and adjustment for age at calving in Nili-Ravi buffaloes. Asian Australasian Journal Animal Science 10:505-509.

Khan, M. S., & Chaudhry, H. Z. (2000). Lactation length and its behavior in Nili-Ravi Buffaloes. Pakistan Veterinary Journal 20 (2).

Khosroshahi, Z. T., Rafat, S. A., & Shoja, D. (2011). Effects of non-genetic factors in milk production and composition in East Azarbaijan native buffaloes of Iran. Buffalo Bulletin 30(3):202-209.

Koç, A., & Kızılkaya ,K. (2009). Factors influencing test day milk yield of Holstein-Friesian Cows. Animal Production 50(1): 24-30.

Koçak, S., Tekerli, M., Çelikeloğlu, K., Erdoğan, M., Bozkurt, Z., & Hacan, Ö. (2019). An investigation on yield and composition of milk, calving interval and repeatabilities ın riverine buffaloes of Anatolia. The Journal of Animal & Plant Sciences 29(3): 650-656.

Kul, E., Şahin, A., Çayıroglu, H., Filik, G., Uğurlutepe, E., & Öz, S. (2016). Effects of Calving Age and Season on Some Milk Yield Traits in Anatolian Buffaloes. Scientific Papers Series D, Animal Science Vol. LIX: 33-37.

Marai, I. F. M., Daader, A. H., Soliman, A. M., & El-Menshawy, S. M. S. (2009). Non-genetic factors affecting growth and reproduction traits of buffaloes under dry management housing (in sub-tropical environment) in Egypt. Livestock Research for Rural Development 21 (3)

Minitab. (2017). Minitab statistical software version 18.1.

Pawar, H. N., Ravi Kumar, G. V. P. P. S., & Raman Narang, R. (2012). Effect of year, season and parity on milk production traits in Murrah buffaloes. Journal of Buffalo Science 1: 122-125.

Rathod, A., Vaidya, M., & Ali, S. (2018). Genetic studies of productive and reproductive attributes of Surti buffalo in Maharashtra. International Journal of Livestock Research 8(8): 309-314.

Rosati, A., Van Vleck, L. D. (2002). Estimation of genetic parameters for milk, fat, protein and mozzarella cheese production for theItalian river buffalo Bubalus bubalis population. Livestock Production Science 74:185–190.

Soysal, M. İ., Guven, H., Gurcan, E. S., & Genc, S. (2015). The Estimation of lactation milk record with partial milk record in Anatolian water buffalo. 8th Asian Buffalo Congress, p.67, April 21-25, Istanbul-Turkey.

Soysal, M. İ., Genç, S., Aksel, M., Özkan Ünal, E., & Gürcan, E. K. (2018). Effect of Environmental Factors on Lactation Milk Yield, Lactation Length and Calving Interval of Anatolian Buffalo in Istanbul. Journal of Animal Science and Products 1 (1):93-101.

Şahin, A., & Ulutaş, Z. (2014). Some environmental factors effect on milk yield estimated with different methods in Anatolian buffaloes. Journal of the Faculty of Veterinary Medicine Kafkas University 20(1):79-85.

Şekerden, Ö. (2011). Factors Affecting Somatic Cell Counts and Their Relations with Milk and Milk Constituent Yield in Anatolian and F1 Anatolian x Italian Crossbred Buffaloes. Animal Production 52 (1): 9-16.

Tekerli, M., Küçükkebabçı, M., Akalın, N. H., & Koçak, S. (2001). Effects of environmental factors on some milk production traits, persistency and calving interval of Anatolian buffaloes. Livestock Production Science 68: 275–281.

Tekerli, M., Altuntaş, A., Birdane, F., Sarımehmetoğlu, O., Doğan, İ., Bozkurt, Z., Erdoğan, M., Çelik, A., Koçak, S., Gürler, Z., Bülbül, T., Kabu, M., Çelikeloğlu, K. (2016). Comparative Determination of Improvement Opportunities for Production Traits, Body Measurement and Protein Polimorphism in a Herd Consisting of Anatolian Buffaloes from Different Regions: Lactation Traits and Genetic Polimorphism. Lalahan Hayvancılık Araştırma Enstitüsü Dergisi 56 (1):7-12.

Tekerli, M. (2019). Buffalo Star data records, account and project tracking program, v5.04. Afyon Kocatepe University, Afyon karahisar, Turkey http://88.249.41.173:83/iletisim.aspx

TUIK. (2020a). Turkish Statistical Institute Statistics. Available in: http://www.turkstat.gov.tr/Start.do, Accessed on: 15 Apr, 2020

TUIK. (2020b). Turkish Statistical Institute Statistics. Available in: http://tuik.gov.tr/PreTablo.do?alt_id=1002, Accessed on: 15 Apr, 2020

Uğurlu, M., Kaya, İ., & Saray, M. (2016). Effects of some environmental factors on calf birth weight and milk yield of Anatolian Water Buffalo (Bubalus Bubalis). Bulgarian Journal of Agricultural Science 22 (6): 995–998.

Livestock Studies, 2020; 60 (2): 62-67
http://doi.org/10.46897/livestockstudies.712627

RESEARCH PAPER

LIVESTOCK STUDIES

Some morphological and physiological characteristics of South Karaman Sheep: I- Morphological features, body measurements and live weights

Dilek Tüney Bebek[1], Mahmut Keskin[2*]

[1] Mustafa Kemal University, Graduate School of Natural and Applied Sciences Antakya, Hatay, Turkey.
[2] Mustafa Kemal University, Department of Animal Science, Faculty of Agriculture Antakya, Hatay, Turkey.

Article History

Received: 01 April 2020
Accepted: 05 October 2020
First Online: 12 October 2020

Corresponding Author

Tel.: 0 (326) 245 5845-1336
E-mail: mkeskin@mku.edu.tr

Keywords

Colour
Horn
Tail type
Body measurement
Live weight

Abstract

In this study, it was aimed to determine the morphological features, body measurements and weights of South Karaman sheep, which is preferred especially by nomadic breeders in Mersin. The animal material of the study consisted of 100 head South Karaman sheep raised in the nomadic system in Tarsus district of Mersin province. The sheep which remained in the tent in the Bahşiş village that is neighbour of the Tarsus district of Mersin province in winter period, and then they migrated to the Çilnili Lake, which is located within the borders of Çamlıyayla district of Mersin province from the beginning of June to the end of October. In the study, morphological features were determined by observation, and body measurements and weights were determined by measuring. At the end of the study, it was determined that the South Karaman sheep are generally black or blackish ash colour. Males of this breed are usually horned and females are hornless. It was also determined that there is an "S" shaped structure at the end of the fat tail in South Karaman sheep.

Introduction

Turkey with the high size of sheep, goats and the cattle population is among the leading countries in the world. However, it can be said that sheep population of Turkey decreased by 16.7% in the last quarter century although it has increased in recent years (Hayvansal Üretim, 2018). This decrease is mainly due to the incentives or supports applied to dairy cattle breeding, reducing in pasture areas and terrorism problems. The fact that sheep breeders prefer to be intensive cattle raising has increased milk production in Turkey and caused serious problems in meat production. At this point, since the climate and pasture characteristics are not suitable for dairy cattle in most of the country the increasing feed prices cannot cover the cost of milk. It is known that Turkey's pastures that usually has poor quality are suitable for sheep rather than cattle. In sheep breeding, there are both breeds with high genetic potential suitable for intensive production and domestic breeds with low yield capacity suitable for traditional production.

Although not involved in the Turkish Statistical Institute data, we know that the different breeds are raised in different regions of Turkey. For example, White Karaman, Red Karaman, South Karaman, Sakız, Awassi, Hamdani, Herik and Merino sheep are bred in Mersin. In the region where nomadic sheep breeding is still widespread, the nomadic breeders prefer the South Karaman sheep (named as Güney Karaman in Turkish) mostly because it is resistant to natural conditions (Bebek and Keskin, 2018).

There is not much information about South Karaman sheep in the literature. However, it can be said that the South Karaman sheep is a breed that is under threat of extinction in terms of pure breeding. Although it is

evaluated this breed is located in the Mediterranean region covering the provinces of Antalya, Mersin, Adana, Gaziantep and Hatay (Özcan, 1989), it is estimated to be the most common in and around Mersin. These sheep, which are accepted as a variant of Karaman sheep (Özcan, 1989) are generally raised in a nomadic system. It is mostly raised as herds by nomads named as Yörük. Although it has been pointed out in various sources that it exists in our country, it is seen that there are not sufficient and detailed studies on the morphological and physiological characteristics of the South Karaman sheep. In this study, it was aimed to determine the morphological features, body measurements and weights of South Karaman sheep.

Materials and Methods

The animal material of the study consisted of 100 head female and 10 head male South Karaman sheep raised in the nomadic system in Tarsus district of Mersin province. The flock was managed under breeders' condition. The herd remained in a tent in the district of Tarsus, of Mersin province (36 ° 46 'North and 34 ° 54' East) from 01 November 2017 until 30 May 2018. After this date, they migrated to the Çinili Lake (37 ° 38 'North and 34 ° 51' East) located in the borders of Çamlıyayla district of Mersin, with an altitude of approximately 2500 m. They returned to Tarsus again on October 20, 2018.

In addition to the pasture, 60 kg of straw and 50 kg of concentrate feed were given to the animals during their stay in Tarsus (40 heads primiparous, 52 heads multiparous, 4 heads infertile and 4 heads aborted). The feeding of animals was provided only by grazing in the pasture during the highland period.

One ram was used for 10 head female animals in mating period. The rams were constantly kept in the herd and the dates they mated were recorded. If a sheep did not show oestrus after mating, it was accepted that she became pregnant. The births started from November 2017 and continued until April 22, 2018.

Body length, withers height, rump height, front chest width, front chest depth and chest circumference were determined as specified by Boztepe *et al.* (1997). All ewes were weighed both on the last 30-45 days of pregnancy and the day after birth. Fleece colour, ear structure, horn condition, horn structure and tail structure were determined by evaluating the animals one by one.

The mathematical model for body weight and sizes is as follows;

$Y_{ij} = \mu + \alpha_i + e_{ij}$

Y_{ij}, recorded value of the ewes in the i^{th} age group

μ, mean of the population

α_i, effect of age groups

e_{ij}, error term

Statistical analysis of the study was evaluated with SPSS package program (SPSS Statistic 17.0.Ink).

Results and Discussion

Morphological Characteristics

Morphological features are features related to colour, shape, size of size and type, which can be seen immediately when viewed from the outside. Each breed has its own colour and even pattern. In this study, it was determined that South Karaman sheep are usually black or blackish ash colour (69%), but also dark brown and pied individuals can be found (Figure 1, 2, 3 and 4). It has been reported by various researchers that South Karaman sheep is generally black in color, and it is seen in brown, ash, white and pied colours (Özcan, 1989; Öztürk, 2000). Özcan (1989) reported that black colour turned into ash colour with the advancement of age in South Karaman sheep as in Karakul sheep. In the present study, it was determined that the South Karaman sheep have medium length and drooping ears and the males are horned. The spiral shaped and curved forward horn can be seen in Figure 1 and the short and thin horn can be seen in Figure 2 in the rams.

It is observed that the females are generally hornless in the South Karaman sheep (Figure 3). In the study, the rate of horned females was determined as 3%. Özcan (1989) reported that male horned females rarely horned in South Karaman sheep. In Karagül sheep, which are considered to be related with South Karaman sheep, male individuals are horned and female individuals are hornless (Uğur, 2006).

Figure 1. South Karaman ram with spiral horns.

Figure 2. South Karaman ram with short and thin horns.

Figure 3. South Karaman female sheep.

Figure 4. View of the tail structure in the sheared South Karaman sheep.

In the study, it was determined that there is an S-shaped structure at the end of the tails of the sheep. The "S" shaped structure at the tip of the tail in the South Karaman sheep with fat tail is one of the distinctive features of this breed (Figure 4).

In the paper on the Registration of Domestic Animal Breeds and Lines published by the Ministry of Agriculture and Forestry, it is stated that the South Karaman sheep are fatty tailed and the tip of the tail hangs down in the form of fingers or "S" (Yerli Hayvan Irk ve Hatlarının Tescili Hakkında Tebliğ, 2004; Küçükbaş Hayvan Seçimi, 2013; Cografya Dünyası, 2014). As stated also by Hunter (2015), the tail end in an "S" shaped formation in Karagül (Karakul) sheep.

South Karaman is the most preferred breed by nomadic breeders in Mersin region because it is suitable for nomadic animal husbandry (Bebek and Keskin, 2018). Because they are resistant to difficult conditions, nomadic breeders prefer to breed South Karaman as pure as possible. The fact that South Karaman sheep is preferred in sheep breeding with nomadic system in Mersin region by different researchers (Aydın and Keskin, 2018; Bebek and Keskin, 2018; Karagöl and Keskin, 2018) indicate that the system is sustainable with native breeds despite various difficulties. The migration of South Karaman sheep from the sea level to the high plateaus and continuing its life in conditions that can be called extensively in areas with very different altitudes causes this breed to be preferred by the breeders. Other reasons why nomadic breeders prefer this breed are the fact that they give a lamb per year (breeder do not want many births because of low milk yield of dams), they are resistant to diseases, and have long walking ability due to nail structure. Özcan (1989) stated that the South Karaman sheep, which has high tolerance to heat and cold, has been grazing up to 2000-2500 m altitude in spring and returned to the seaside in autumn. Ertuğrul *et al.* (2005) reported that South Karaman sheep, like all domestic breeds, are very well adapted to insufficient environmental conditions as they are bred in the region for many years. This breed is both durable and that they can birth even under insufficient environmental conditions. Karakul sheep, considered to be related to Southern Karaman sheep, are also extremely resistant to harsh conditions and can live in desert conditions and consume saltwater (Hunter, 2015).

Body Measurements and Live Weights

Body measurements and live weights are important features that can be used to differentiate breeds and are therefore used in breed definitions. From these measures, especially live weight can change before and after birth (Table 1). Also, since the animal can continue to de-

velop after giving birth for the first time, the age of the animal may also have an effect on body measurements and weights (Table 2).

Table 1. Variation of live weights with birth type in South Karaman sheep ($\bar{x}$ ± se)

Traits	Single (60)	Twin (32)	P	Total
Live weight[1]	50.6±0.87	58.9±0.99	<0.01	53.2±0.77
Live weight[2]	44.6±0.86	49.3±0.94	<0.05	46.1±0.69

Live weight[1], live weight in the last 30-45th days of gestation; Live weight[2], live weight after birth; $\bar{x}$, mean; se, standard error

As can be seen from Table 1, the average body weight of ewes taken after birth was 50.6 ± 0.87 and 58.9 ± 0.99 kg in the single and twin births, respectively (P <0.01). It was determined that the mean body weight difference, which was 8.3 kg between the single and twin births, decreased to 4.7 kg by decreasing in weighting made after birth, and the live weights for both groups were 44.6 ± 0.86 and 49.3 ± 0.69, respectively. It is normal for the sheep that give twin birth have higher prenatal body weight than those who give single birth when maintenance and feeding are sufficient (Demirel *et al.,* 2000). Effect of the age of the experimental ewes on their body weight and body measurements are seen in Table 2. These findings show that the growth and development for South Karaman ewes continues after the age of 2. These values determined in the experimental South Karaman sheep are similar to the values reported by different researchers (Ayhan, 2015; Yılmaz *et al.,* 2013). Thus, Ayhan (2015) reported wither height, body length, and adult body weight as 63 cm, 58 cm and 37 kg, respectively for South Karaman sheep. Same way, Yılmaz *et al.* (2013) informed these values for same characters as 63 cm, 58 cm and 47 kg, respectively. Akay *et al.* (2018) reported chest circumference, height of wither, height of rump, body length, width of front chest and width of rump in South Karaman ewes as 84.49 cm, 62.95 cm, 62.72 cm, 61.55 cm, 17.51 cm and 18.87 cm, respectively. It is seen that the values determined in the current study are compatible with the body size values reported by Akay *et al.* (2018). The differences between the values of body measurements determined in the present study and those reported for Karakul sheep by Erol and Akçadağ (2009) may have caused from the differences in breeding conditions and breeds.

Table 2. Change of body weight and body size in experimental sheep according to age ($\bar{x}$ ± se)

Traits	18-24 months (n=40)	36 months and up (n=52)	P	Total
Body weight[1]	47.5±1.06	57.1±0.75	<0.01	53.2±0.77
Body weight[2]	41.0±0.90	49.3±0.70	<0.01	46.1±0.69
Wither height	63.0±0.33	64.5±0.27	<0.01	63.9±0.22
Rump height	64.9±0.36	66.4±0.28	<0.01	65.8±0.23
Body length	58.7±0.67	61.5±0.46	<0.01	60.4±0.41
width of front chest	19.6±0.24	20.7±0.20	<0.01	20.3±0.20
Chest circumference	99.1±1.11	106.7±0.79	<0.01	103.6±0.75

Live weight[1], live weight in the last 30-45th days of gestation; Live weight[2], live weight after birth; $\bar{x}$, mean; se, standard error

Relationships Between Body Weight and Body Measurements

The relationship between live weight and different body measurements, especially chest circumference, in sheep and goats is expressed by different researchers (Gül *et al*,, 2005; Koç and Akman, 2007; Şahin *et al.,* 2018). These relations allow to using of the size of the chest circumference, which is more practical than weighting the animal, in market conditions where the animal trade is based on live weight.

The correlation coefficients and the statistical significance levels between the different body measurements and live weight values determined from the experimental sheep are given in Table 3. Although there were significant (P <0.01) correlations between the features mentioned in Table 3, regression equations have been created for the relationship between chest circumference and body weight, which have the highest correlation coefficient and practical usage.

Accordingly, the regression equations between body weight and chest circumference for gestational period and after birth period were formulated as “Live weight= -23.5+0.74 x Chest circumference” and “Live weight = -17.1+0.61 x Chest circumference”, respectively.

The correlation coefficient between these two properties for same periods were calculated as 0.711 and 0.658, respectively (P <0.01). Live weights estimated by the calculated regression equation were given in Table 4.

Table 3. Correlation coefficients between different body sizes and weights

	LW[1]	LW[2]	WH	RH	BL	FCW	CC
LW[1]	1	0.964**	0.343**	0.415**	0.354**	0.577**	0.711**
LW[2]		1	0.312**	0.357**	0.343**	0.539**	0.658**
WH			1	0.791**	0.504**	0.321**	0.376**
RH				1	0.537**	0.387**	0.440**
BL					1	0.289**	0.360**
FCW						1	0.654**
CC							1

LW[1],live weight in the last 30-45th days of gestation; LW2, live weight after birth; WH, Wither height; RH, Rump height; BL, Body length; FCW, width of front chest; CC, chest circumference; **, P<0.01

Table 4. The relationship between live weight during pregnancy and chest circumference

Last 30-45th days of gestation										
CC	90	92	94	96	98	100	102	104	106	108
LW	43.1	44.6	46.1	47.5	49.0	50.5	52.0	53.5	54.9	56.4
After Birth										
GÇ	90	92	94	96	98	100	102	104	106	108
CA	37.8	39.2	40.2	41.5	42.7	43.9	45.1	46.3	47.6	48.8

CC, chest circumference, LW, Live weight

This type of correlation and regression study has not been found in the literature in South Karaman ewes. However, they were reported correlations between live weight and body measurements ranging from 0.674 to 0.788 (P <0.01), and expressed the regression equation between live weight and chest circumference as "live weight = -51.8 + 1.04 chest circumference" for Anatolian Merino ewes by Şahin *et al.* (2018).

Conclusions

The production purpose in sheep breeding can vary according to the countries. As concepts such as global climate change and organic production, became widespread in Turkey the importance of native breeds raised for both milk and meat is better understood. In addition, considering the suitability of nomadic breeding, South Karaman sheep is an important local gene source. In this study, the followings have been stated as conclusions; (a) this breed has generally black or blackish ash and dark brown colours, males were horned and females were hornless, has a fatty tail with an "S" shaped extension at the end, (b) there were statistically significant correlations between different body measurements and body weight scales and the highest correlation coefficient was determined between body weight and chest circumference, (c) Live weight can be estimated safely by measuring the chest circumference in the establishment where it is not possible to weigh the sheep.

Acknowledgements

This manuscript has been prepared as part of a PhD thesis entitled as "Some Morphological and Physiological Features of South Karaman Sheep". The authors thank Coordinatorship of Scientific Research Projects of Hatay Mustafa Kemal University (Project No: 17.D.002) which provides financial support to the study.

References

Akay, N., Yılmaz, T. C. & Yılmaz, O. (2018, May 09-12). Live weight estimation based on linear body measurements of South Karaman sheep breed, [Paper presentation], I. International Agricultural Science Congress, Van/Turkey.

Yerli Hayvan Irk ve Hatlarının Tescili Hakkında Tebliğ. (2004). https://www.mevzuat.gov.tr/mevzuat?MevzuatNo=6109&MevzuatTur=9&MevzuatTertip=5 Tebliğ no:2004/39. Resmi Gazete Tarihi: 12.12.2004, sayısı: 25668.

Küçükbaş Hayvan Seçimi. (2013). T.C. Milli Eğitim Bakanlığı, Hayvan Yetiştiriciliği, Küçükbaş Hayvan Seçimi. http://docplayer.biz.tr/12421685-Hayvan-yetistiriciligi.html

Cografya Dünyası. (2014). Mersin Ekonomik Faaliyetler, http://www.cografya.gen.tr/tr/mersin/ ekonomi.html

Hayvansal Üretim. (2018). http://www.tuik.gov.tr/UstMenu.do?metod=temelist

Aydın, M. K. &, Keskin, M. (2018). Muğla ilinde küçükbaş hayvan yetiştiriciliğinin yapısal özellikleri. Mediterranean Agricultural Sciences, 31: 317-323.

Ayhan, V. (2015). Koyun Keçi Irklarımız Tanıtım Kılavuzu. Türkiye Damızlık Koyun Keçi Yetiştiricileri Merkez Birliği, Yayın No: 5, Ankara.

Bebek, D. T. & Keskin, M. (2018). Mersin ilinde koyun yetiştiriciliğinin mevcut durumu bazı verim ve yapısal özellikleri. Mustafa Kemal Üniversitesi Ziraat Fakültesi Dergisi, 23(2): 315-323.

Boztepe, S., Dağ, B., Parlat, S. S., Yıldız, A. Ö. & Aktaş, A. H. (1997). Yağlı kuyruklu kimi yerli ırk kuzuların besi performansı ve karkas özellikleri. Selçuk Üniversitesi Araştırma Fonu. Proje No: ZF–95/064. Konya

Demirel, M., Aygün, T., Altın, T. & Bingöl, M. (2000). Hamdani ve Karakaş koyunlarında gebeliğin son döneminde farklı düzeylerde beslemenin koyunlarda canlı ağırlık, kuzularda doğum

ağırlığı ve büyüme üzerine etkileri. Turkish Journal of Veterinary and Animal Sciences, 24: 243–249.

Erol, H. & Akçadağ, H. İ. (2009). Halk elinde yetiştirilen Karagül koyun sürülerinde bazı verim özellikleri. Lalahan Hayvancılık Araştırma Enstitüsü Dergisi, 49(2): 91-104.

Ertuğrul, M., Dellal, G., Elmacı, C., Akın, O., Karaca, O., Altın, T. & Cemal, İ. (2005). Hayvansal gen kaynaklarının koruma ve kullanımı. https://www.zmo.org.tr/resimler/ekler/92cb13c22d51c22_ek.pdf?tipi=14&sube=

Gül, S., Görgülü, O., Keskin, M., Biçer, O. & Sarı, A. (2005). Some prediction equations of live weight from different body measurements in Shami (Damascus) goats. Journal of Animal and Veterinary Advances, 4(5) : 497-501.

Hunter, D. Y. (2015). Karakul sheep. The Livestock Conservancy News. Spring. 2015, page 6-9. https://karakulsheep.files.wordpress.com/2016/01/karakul-sheep-lc-news-spring-2015.pdf.

Karagöl, E. & Keskin, M. (2018). Problems of nomadic goat breeders and their effects on forest. Mugla Journal of Science and Technology, 4(1): 11-15.

Koç, A. & Akman, N. (2007). Siyah-Alaca tosunların değişik dönemlerdeki vücut ölçüleri ve vücut ölçülerinden canlı ağırlığın tahmini. ADÜ Ziraat Fakültesi Dergisi, 4(1-2): 21 – 25.

Özcan, L. (1989). Küçükbaş Hayvan Yetiştirme-II. Çukurova Üniversitesi Ziraat Fakültesi Yayınları No: 108, Adana.

Öztürk, A. (2000). Koyunculuk ve Yapağı. Selçuk Üniversitesi Zootekni Bölümü Ders notları. Konya.

Şahin, Ö., Boztepe, S. & Keskin, İ. (2018). Anadolu Merinosu erkek kuzularında besi dönemi vücut ölçülerine ait ortalamalardan canlı ağırlık, canlı ağırlık artışı ve yem tüketiminin tahmini. Selcuk Journal of Agriculture and Food Sciences, 32(2); 142-145.

Uğur, F. (2006). Genel Hayvan Yetiştirme, Ders Notu. Çanakkale Onsekiz Mart Üniversitesi Ziraat Fakültesi Zootekni Bölümü.

Yılmaz, O., Cengiz, F., Ertuğrul, M. & Wilson, R. T. (2013). The domestic livestock resources of Turkey: Sheep breeds and crossbreeds and their conservation status. Animal Genetic Resources, 52: 147-163.

Livestock Studies, 2020; 60 (2): 68-73
http://doi.org/10.46897/livestockstudies.846425

RESEARCH PAPER

LIVESTOCK STUDIES

Evaluation of two SNP markers in DPPA2 and SYTL3 genes for association with host response against Visna/Maedi infection in Turkish sheep

Yalçın Yaman[1]

[1] Sheep Breeding and Research Institute Bandirma, Balikesir, Turkey.

Article History

Received: 06 March 2020
Accepted: 13 November 2020
First Online: 25 December 2020

Corresponding Author

Tel.: +902667380081/ 1246
E-mail: yalcinyaman@gmail.com

Keywords

Visna/maedi
Genetic association
Genetic resistance/susceptibility

Abstract

Visna/maedi (VM) is an incurable viral disease of sheep causing serious production losses across the globe. Classical control measures against VM such as screening and culling are costly and time-consuming. Breeding VM resistant sheep could provide an opportunity for struggling with the VM and decreasing the economic loss. In this study, we aimed to investigate possible associations between two previously reported single nucleotide polymorphisms (SNPs) in the ovine DPPA2 and SYTL3 genes and VM serostatus, and evaluate implementation of selective breeding strategies against VM in Karacabey merino, Kivircik, Imroz, and composite breeds; Bandirma, Hampshire crosses (HAMP), Ramlic and Black-headed German mutton crosses (SBA) which are reared in Marmara region of Turkey. For this purpose, we genotyped the sheep which VM serostatus were determined previously. The genotyping results showed that these SNPs in the DPPA2 and SYTL3 genes are polymorphic. We have conducted an association analysis with an experimental design using case-control matched pairs. Finally, a power analysis was performed to determine the power of the statistical analysis. According to our findings, within our detection limits (the minimum odds ratio 2.5 to 2.8; CI 95; statistical power 0.96; p-value < 0.05), there was no significant association between the SNPs in the DPPA2 and SYTL3 genes and VM serostatus. Therefore, these SNP markers are not useful to selective breeding against VM in Turkish sheep.

Introduction

Visna/maedi (VM) is a viral infection in sheep caused by lentiviruses and characterized with a long incubation period, slow progression, weight loss and eventually death. Although it is a multisystemic disease, there are two main manifestations of VM: visna (progressive inflammation of the central nervous system) and maedi (respiratory form characterized by interstitial pneumonia). Because of pathogenic and genetic similarity between VM virus and Caprine arthritis encephalitis virus (CAEV), both viruses called to be small ruminant lentiviruses (SRLV) (Gomez-Lucia *et al*., 2018). Furthermore, VMV share common features such as genome organization, virus replication mode, and latency with HIV virus that is causative agent of Acquired immunodeficiency syndrome (AIDS) in humans (Andrésdóttir, 2018). The main transmission route of VMV is from mother to lamb via colostrum and milk, however aerosol route either in close contact or up to several meters is another common mode of transmission (Peterhans *et al*., 2004). There is no effective treatment and also a commercial vaccine not available for VM (Gomez-Lucia *et al*., 2018), thus, disease control strategies generally are based on serological screening and culling infected animals (Pépin *et al*., 1998).

VM is distributed in sheep industry across the globe and responsible for serious production loss. VM prevalence have been reported as 24.8% in Spain (Lago *et al*., 2012), 71% in Lebanon (Tabet *et al*., 2017), from 10.5 to 21.6% in Ethiopia (Ayalet *et al*., 2001), 28.8% in Germany (Huttner *et al*., 2017), from 20 to 60% in the UK (Ogden *et al*., 2019), 34.8% in Kosovo (Cana *et al.,* 2020), and

from 3.3 to 96.7% in Canada (Heinrichs *et al*., 2017). In Turkey prevalence of VM was reported between 2.7 to 77.9% (Burgu and Toker, 1990; Yavru *et al*., 2012; Muz *et al*., 2013).

Due to no available treatment and/or immunization to struggle with VM, efforts turned towards genetic research to identify the underlying host genetic factors against VM. Various studies have proposed a number of candidate loci to be associated with VM disease status (Herrmann-Hoesing *et al*., 2008; White *et al*., 2009; Larruskain *et al*., 2010; Sarafidou *et al*., 2013). However, a major gene (TMEM154) was reported to be associated with host susceptibility/resistance to VM in a genome-wide association (GWA) study using case-control design (Heaton *et al*., 2012), and this result was confirmed by subsequent independent studies (Molaee et at., 2018; Molaee *et al*., 2019; Yaman *et al*., 2019). Moreover, SNP markers in the ovine DPPA2 and SYTL3 genes were reported by another GWA study to be potential co-receptors for VM infection White *et al*., 2012).

The aim of present study was to investigate whether there is an association between SNP markers in the DPPA2 and SYTL3 genes and VM serostatus in Turkish sheep. To this end, a retrospective cohort study was performed to determine serostatus of sheep which have been reared at Sheep Breeding and Research Institute (SRI) in the same environmental and management conditions. A case-control matched pairs panel was constructed and samples were genotyped using single nucleotide primer extension (SNuPE) assay. Finally, a McNemar's test (McNemar, 1947) for correlated proportions was conducted to determine any significant association between SNPs of interest and VM serostatus in Turkish sheep.

Material and Methods

Animals

Native Turkish sheep; Karacabey merino, Kivircik, Imroz, and composite breeds; Bandirma, Hampshire crosses (HAMP), Ramlic and Black-headed German mutton crosses (SBA) were used to study of which serological VM status were previously determined with indirect-ELISA in 2017 (Yaman *et al*., 2019). All sheep were from a research flock that have been bred in SRI. For genetic analysis, a tube of peripheral whole blood with EDTA was collected from V. jugularis in aseptic conditions. Sampled animals were two years old or older. A case-control matched pairs panel was constructed, and genetic analysis performed on matched pairs.

DNA isolation was conducted using commercial spin-column kits according to the manufacturer's manual. Primers were designed using primer blast online tool (https://www.ncbi.nlm.nih.gov/ tools/primer-blast/). A multiplex polymerase chain reaction (PCR) was employed to amplify the regions of the ovine DPPA2 and STYL3 genes covering the target SNPs. Single nucleotide primer extension (SNuPE) experiment was designed to genotype target SNPs at the same time. Briefly, extension primers without fluorescent tag were designed for each SNP in different lengths (18 vs 26bp) to bind one base prior to the target SNP. Then, SNaPshot™ Multiplex Kit (Thermo Fisher Scientific Inc., USA) was used for SNuPE assay in standard thermal cycler. Throughout the SNuPE reaction, it was expected that the fluorescently labeled ddNTPs bind exactly to the target nucleotide and chain termination reaction occurs. Finally, after incubation with shrimp alkaline phosphatase (SAP) for enzymatic purification, reaction products were subjected to capillary electrophoresis with fragment analysis protocol on ABI 3500 sequencer platform. Amplification primers, extension primers and a summary of the genotyped SNPs are provided in Table 1. Chromatograms were visualized using GeneMapper v6 software. To confirm the SNuPE results, approximately 10% of the samples were sequenced for each SNP.

Genetic association studies require maximum control of other factors, particularly for disease traits. Exposure intensity and exposure duration are two major factors affecting the disease status. Additionally, breed effect (population stratification or population structure) is another major factor on the results of association analyses. To account for exposure duration, exposure intensity and breed effect, case-control matched pairs were constructed. Briefly, a seropositive ewe matched with a seronegative from the same breed (for breed effect), the same age (for exposure duration) and the same flock (for exposure intensity), and statistical analysis was performed over case-control matched pairs. For DPPA2, 127 matched pairs (127 case and 127 control; n= 254) and for STYL3, 131 matched pairs (131 cases, 131 controls; n= 262) were constructed. Matched pairs panel according to breed and ages are given in Table 2. To determine whether there is any association between interested SNPs and VM serostatus a McNemar's test for correlation proportion was conducted. Association analysis was performed for three heritability model:1-exactly one copy of allele provides genetic risk or protection, 2-one or two copies of allele provides genetic risk or protection, and 3- exactly two copy of allele provides genetic risk or protection. Matched pairs were assigned to be (1;1), (1;0), (0;1), and (0;0) where in (1;1) pairs, either case and control members of the pair have the risk/protection factor, in (1;0) pairs, the case has the risk/protective factor but control does not, in (0;1) pairs, the case does not have the risk/protection factor

but the control has, and in (0,0) pair, neither of the case nor the control have the risk/protection factor. Assigned pairs were manually arranged and McNemar's test was perfomed an online tool (https://www.graphpad.com/quickcalcs/McNemar1.cfm) using number of each assigned pairs. It is expected for a significant association, the sum of discordant pairs (1;0 and 0;1) must be greater than 25. Finally, a power analysis using G*Power v3.1.9.4 (Faul *et al*., 2009) software was conducted to check the statistical power of the study for each SNP marker.

Table 1. A summary of amplification and extension primers

Primer ID	Amplification primers	PCR Size	Extension primers	Size (bp)	SNP	rs
styl3-F	GCTTCTCAATTCCGCCCTTTC	791	CTTTGAAGACGGCTGCTT	18	A/C/T	rs413063847
styl3-R	CTAGGCGCTATGGTGAGCTG					
dppa2-F	TGAAGTTACCACCTCAACCGT	884	GTGATGATTTAGGAATAT ACTGCAAA	26	C/T	rs411941451
dppa2-R	GATCTCTGGTGCTTGGAACA					

Table 2. Distribution of matched pairs according to breeds and ages

	DPPA2								STYL3							
	Ages								Ages							
Breeds	8	7	6	5	4	3	2	Total	8	7	6	5	4	3	2	Total
Karacabey merino	-	2	3	2	3	4	1	15	-	3	2	2	3	3	1	14
Kivircik	-	7	11	9	7	4	-	38	-	9	12	9	7	4	-	41
Imroz	-	-	6	6	2	2	1	17	-	-	6	6	2	2	1	17
Bandirma	3	11	11	14	9	3	-	51	4	13	12	14	8	3	-	54
HAMP	-	-	2	-	1	-	-	3	-	-	2	-	1	-	-	3
SBA	-	-	-	-	-	2	-	2	-	-	-	-	-	2	-	2
Ramlic	-	-	1	-	-	-	-	1	-	-	-	-	-	-	-	-
Total	3	20	34	31	22	15	2	127	4	25	34	31	21	14	2	131

Results

SNuPE assay results revealed that each SNP marker in the DPPA2 and STYL3 genes were polymorphic for all breeds except Ramlic. Only two matched pair were available from Ramlic ewes for the SNP in DPPA2 gene, nevertheless these four sheep were monomorphic regarding this SNP. Minor allele frequency (MAF) for the SNP in DPPA2 ranked from 0.13 (SBA) to 0.50 (Imroz) and for the SNP in STYL3 ranked from 0.25 (SBA) to 0.44 (Imroz, Table 3). For the SNP in DPPA2 in Imroz and Ramlic and for the SNP in STYL3 in Karacabey merino and Hampshire crosses minor alleles have turned to be a major allele. Sequence results for 10% of the samples for each SNP were in 100% concordance with SNuPE assay.

Table 3. Allele distribution of DPPA2 and STYL3 SNPs according to breeds

	DPPA2				STYL3			
Breeds	*n*	HW	MAF	Alleles	*n*	HW	MAF	Alleles
Karacabey merinos	30	0.52	0.20	T/C	28	1.00	0.42	T/C
Kivircik	76	0.53	0.35	T/C	82	0.42	0.43	C/T
Imroz	34	0.44	0.50	C/T	34	1.00	0.44	C/T
Bandirma	102	0.84	0.24	T/C	108	0.36	0.27	C/T
HAMP	6	1.00	0.08	T/C	6	0.48	0.42	T/C
SBA	4	1.00	0.13	T/C	4	1.00	0.25	C/T
Ramlic	2	1.00	-	C/T	-	-	-	0
Total	254				262			

McNemar's test for correlated proportion revealed that number of discordant pairs were greater than 25 for all three scenario (exactly one allele, one or two allele, and exactly two allele provide risk or protection factor) except "one or two allele" model for STYL3 gene. Statistical power analysis was performed over real percent of discordant pairs and sample size. Detection limits of the study were determined to have statistical power as 0.96, minimum odds ratio as 2.5; CI as 95; p-value < 0.05 for the SNP in DPPA2, and statistical power as 0.96, minimum odds ratio as 2.8, CI as 95; p-value < 0.05 for the SNP in STYL3. Statistical analysis did not indicate any significant association between the SNP markers in DPPA2 and STYL3 with VM serostatus within our statistical limits (Table 4).

Table 4. McNemar's test for VM association with *DPPA2* and *STYL3* SNP markers.

		SNP ID	
McNemars pair status and test statistics[a]	**McNemar's quadrants and equations**[b]	***DPPA2***	***STYL3***
Exactly one copy of risk or protective allele			
1,1	"a"	29	23
1,0	"b"	35	27
0,1	"c"	34	32
0,0	"d"	29	49
Total pairs	a+b+c+d	127	131
Discordant pairs	b+c	69	59
OR	b/c	1.0	0.8
CI95 Lower	-	0.6	0.5
CI95 Upper	-	1.7	1.4
McNemar's $\chi 2$[d]	$(\|b - c\| - 1)^2/(b + c)$	0.0	0.3
p-value	-	0.19	0.17
One or two copies of risk or protective allele			
1,1	"a"	53	105
1,0	"b"	24	10
0,1	"c"	33	13
0,0	"d"	17	3
Total pairs	a+b+c+d	127	131
Discordant pairs	b+c	57	23
OR	b/c	0.7	0.8
CI95 Lower	-	0.4	0.3
CI95 Upper	-	1.2	1.8
McNemar's $\chi 2$	$(\|b - c\| - 1)^2/(b + c)$	1.1	0.2
p-value	-	0.10	0.27
Exactly two copies of risk or protective allele			
1,1	"a"	3	35
1,0	"b"	10	30
0,1	"c"	20	28
0,0	"d"	94	38
Total pairs	a+b+c+d	127	131
Discordant pairs	b+c	30	58
OR	b/c	0.5	1.1
CI95 Lower	-	0.2	0.6
CI95 Upper	-	1.1	1.8
McNemar's $\chi 2$	$(\|b - c\| - 1)^2/(b + c)$	2.7	0.0
p-value	-	0.06	0.20

[a]Each member of a case-control pair is assigned a value of "1" or "0" depending on whether the risk/protective factor is present (1) or absent (0). Briefly, in (1,1) pairs, either case and control members of the pair have risk/protection factor, in (1,0) pairs, case has risk/protective factor but control not, in (0,1) pairs, case not has risk/protection factor but control have, and in (0,0) pair neither of case and control have risk/protection factor.
[b]These are quadrants from the McNemar's contingency table for classifying pairs.

Discussion

VM is among the most prevalent disease in sheep industry worldwide. Only Australia and New Zealand have been considered to be MV free, but not for the CAEV, the goat type of the SRLV. Furthermore, while Japan has also been considered free from MV, recent studies showed the presence of the VM virus in this country (Gomez-Lucia *et al*., 2018). In addition to having no treatment or vaccine, vertical transmission via colostrum from ewe to lamb makes it much more difficult to struggle with VM. Moreover, control measures such as screening, culling and restocking have been found to be expensive and time consuming (Pépin *et al*., 1998; Berriatua *et al*., 2003; Ruiz-Fons *et al*., 2014). Nevertheless, it should be kept in mind that even if MV eradication is achieved by classical control measures, the flocks will remain susceptible against VM, thus, possibility of new infection by vectors or by infected ewes joining the herd from other flocks will remain as a threat for sheep herds.

On the other hand, breeding for VM resistant sheep could provide an opportunity to struggle with VM. For this reason, a variety of research on molecular mechanisms underlying host genetic factors against VM have been conducted. Herrmann-Hoesing et al. (2008) tested the possible association between DRB1 gene in Ovar-MHC II loci and provirus level of Ovine Progressive Pneumonia (OPP) that is counter part of VM, and they found that DRB1*0403 or DRB1*07012 alleles were associated with lower proviros level of OPP (Herrmann-Hoesing et al., 2008). Another report seeking the association between DRB1 and VM status revealed that the DRB1*0325 allele associated with susceptibility to VM (Larruskain *et al*., 2010). White *et al*. (2009) reported that a 4-base deletion in the promoter domain of CCR5 gene significantly reduced the provirus level in homozygous. Another reported deletion variant in ZNF389 gene also reported to be associated with provirus level of VM infection (White *et al*., 2013). Sarafidou *et al*. (2013) proposed that G520R mutation in ovine TLR9 coding region associated with VM serostatus. Nevertheless, there is no published data from independent studies to confirm the effect of these loci.

In a recent study, however, specific haplotypes in TMEM154 gene proposed to be major gene regarding genetic resistance/susceptibility to VM (Heaton *et al*., 2012), and it is repeatedly reported by subsequence studies that TMEM154 variants can explain the big proportion of the VM serostatus variations in North American (Leymaster *et al*., 2013; 2015), German (Molaee *et al*., 2018), Turkish (Yaman *et al.*, 2019), and Iranian (Molaee *et al*., 2019) sheep. But it might be hypothesized that different co-receptors encoded by other genomic locations are quite possible.

In a recent genome-wide association study, White *et al*. (2012) reported that multiple SNPs located within or near the various genes might be involved in host immune response to VM. They have also reported one SNP within DPPA2 and one SNP within STYL3 genes significantly associated with VM status. In the present study, the possible effect of these two SNPs was tested in Turkish sheep with an experimental design including case-control matched pairs. However, the results of White *et al*. (2012) have not been confirmed in Turkish sheep. The reasons for not being confirmed for these results might arise from differences in experimental design of the studies, different subtypes of the VM viruses, and gene-environment interaction.

In conclusion, within our detection limits, no association was detected between VM serostatus and previously reported two SNPs located in the ovine DPPA2 and the ovine STYL3 genes. These SNPs are not useful for selective breeding in Turkish sheep. Further studies might be required to elusive involved co-receptors in the host immune response to the VM virus.

Acknowledgements

This research has been funded by the Republic of Turkey Ministry of Agriculture and Forestry, General Directorate of Agricultural Research and Policies (Project number: TAGEM/HAYSÜD/15/A01/P02/02-02).

Ethical statement

All animal procedures in the study were reviewed and approved by the local ethics committee of Sheep Breeding and Research Institute (Approval Number: 1282412).

Conflict of interests

The author declares no conflicts of interest.

References

Andrésdóttir, V. (2018). Maedi-Visna virus as a model for HIV. Icelandic Agricultural Sciences, 31(1), 23-47.

Ayelet, G., Roger, F., Tibbo, M., & Tembely, S. (2001). Survey of Maedi-Visna (MV) in Ethiopian Highland Sheep. Veterinary Journal, 161(2), 208-210.

Berriatua, E., Alvarez, V., Extramiana, B., González, L., Daltabuit, M., & Juste, R. (2003). Transmission and Control Implications of Seroconversion to Maedi-Visna Virus in Basque Dairy-Sheep Flocks. Preventive Veterinary Medicine, 60(4), 265-279.

Burgu, I., & Toker, A. (1990). Türkiye'de Visna-Maedi Enfeksiyonunun Serolojik Olarak Araştırılması. Ankara Üniversitesi Veteriner Fakültesi Dergisi, 37(3), 538-553.

Cana, A., Taylor, N., Honhold, N., Gjinovci, V., Osmani, A., Nardelli, S., Murati, B., Spahiu, J., Mehmetukaj, D., & Alishani, M. (2020). Sero-Prevalence Survey of Small Ruminant Len-

tivirus (SRLV) Infections in Kosovo. Kafkas Universitesi Veteriner Fakultesi Dergisi, 26(1), 1-8.

Faul, F., Erdfelder, E., Buchner, A., & Lang, A, G. (2009). Statistical Power Analyses Using G * Power 3.1: Tests for correlation and regression analyses. Behavior Research Methods, 41(4), 1149-1160.

Gomez-Lucia, E., Barquero, N., & Domenech, A. (2018). Maedi-Visna virus: current perspectives. Veterinary Medicine: Research and Reports, 9, 11–21.

Heaton, M. P., Clawson, M. L., Chitko-Mckown, C. G., Leymaster, K. A., Smith, T. P., Harhay, G. P., White, S. N., Herrmann-Hoesing, L. M., Mousel, M. R., Lewis, G. S., Kalbfleisch, T. S., Keen, J. E., & Laegreid, W. W. (2012). Reduced Lentivirus Susceptibility in Sheep with TMEM154 Mutations. PLoS Genetics, 8(1): e1002467

Heinrichs, R., Wilkins, W., Schroeder, G., & Campbell, J. (2017). Prevalence of Maedi-Visna in Saskatchewan Sheep. Canadian Veterinary Journal, 58(2), 183-186.

Herrmann-Hoesing, L. M., White, S. N., Mousel, M. R., Lewis, G. S., & Knowles, D. P. (2008). Ovine Progressive Pneumonia Provirus Levels Associate with Breed and Ovar-DRB1. Immunogenetics, 60(12), 749-758.

Huttner, K., Heyne, H., & Heim, D. (2017). Impact of flock segregation according to the Maedi-Visna status on reproduction and lamb rearing - a field study in Mecklenburg-Vorpommern. Berliner und Münchener Tierärztliche Wochenschrift, 130(11-12), 490-493.

Lago, N., López, C., Panadero, R., Cienfuegos, S., Pato, J., Prieto, A., Díaz, P., Mourazos, N., & Fernández, G. (2012). Seroprevalence and risk factors associated with Visna/Maedi virus in semi-ıntensive lamb-producing flocks in northwestern Spain. Preventive Veterinary Medicine, 103(2–3), 163-169.

Larruskain, A., Minguijón, E., García-Etxebarria, K., Moreno, B., Arostegui, I., Juste, R. A., & Jugo, B. M. (2010). MHC Class II DRB1 Gene Polymorphism in the Pathogenesis of Maedi–Visna and Pulmonary Adenocarcinoma Viral Diseases in Sheep. Immunogenetics, 62(2), 75-83.

Leymaster, K. A., Chitko-McKown, C. G., Clawson, M. L., Harhay, G. P., & Heaton, M. P. (2013). Effects of TMEM154 haplotypes 1 and 3 on susceptibility to ovine progressive pneumonia virus following natural exposure in sheep. Journal of Animal Science, 91, 5114–5121.

Leymaster, K. A., Chitko-McKown, C. G., & Heaton, M. P. (2015). Incidence of infection in 39-month-old ewes with TMEM154 diplotypes “1 1,” “1 3,” and “3 3” after natural exposure to ovine progressive pneumonia virus. Journal of Animal Science, 93, 41–45.

McNemar, Q. (1947). Note on the sampling error of the difference between correlated proportions. Psychometrika, 12, 153-157.

Molaee, V., Eltanany, M., & Lühken, G. (2018). First survey on association of TMEM154 and CCR5 variants with serological maedi-visna status of sheep in German flocks. Veterinary Research, 49(1): 36.

Molaee, V., Otarod, V., Abdollahi, D., & Lühken, G. (2019). Lentivirus Susceptibility in Iranian and German Assessed by Determination of TMEM154 E35K. Animals, 9(9), 685.

Muz, D., Oğuzoğlu, T. C., Rosati, S., Reina, R., Bertolotti, L., & Burgu, I. (2013). First molecular characterization of visna / maedi viruses from naturally infected sheep in Turkey. Archives of Virology, 158(3), 559-570.

Ogden, N., Davies, P., & Lovatt, F. (2019). Dealing with maedi visna in UK sheep flocks. In Practice, 41(7), 321-328.

Pépin, M., Vitu, C., Russo, P., Mornex, J. F., & Peterhans, E. (1998). Maedi-visna virus infection in sheep: a review. Veterinary Research, 29(3-4), 341-367.

Peterhans, E., Greenland, T., Badiola, J., Harkiss, G., Bertoni, G., Amorena, B., Eliaszewicz, M., Juste, R. A., Krassnig, R., Lafont, J. P., Lenihan, P., Pétursson, G., Pritchard, G., Thorley, J., Vitu, C., Mornex, J. F., & Pépin, M. (2004). Routes of transmission and consequences of small ruminant lentiviruses (SRLVs) infection and eradication schemes. Veterinary Research, 35(3), 467-483.

Ruiz-Fons, F., González-Barrio, D., Aguilar-Ríos, F., Soler, A. J., Garde, J. J., Gortázar, C., & Fernández-Santos Mdel, R. (2014). Infectious pathogens potentially transmitted by semen of the black variety of the Manchega sheep breed: Health constraints for conservation purposes. Animal Reproduction Science, 149(3-4), 152-157.

Sarafidou, T., Stamatis, C., Kalozoumi, G., Spyrou, V., Fthenakis, G. C., Billinis, C., & Mamuris, Z. (2013). Toll Like Receptor 9 (TLR9) Polymorphism G520R in Sheep Is Associated with Seropositivity for Small Ruminant Lentivirus. PLoS One, 8(5), e63901.

Tabet, E., Tlaige, R., El Hage, J., & Abi-Rizk, A. (2017). The occurrence of maedi-visna virus in Lebanon. Revue Scientifique et Technique, 36(3), 899-903.

White, S. N., Mousel, M. R., Herrmann-Hoesing, L. M., Reynolds, J. O., Leymaster, K. A., Neibergs, H. L., Lewis, G. S., & Knowles, D. P. (2012). Genome-wide association identifies multiple genomic regions associated with susceptibility to and control of ovine lentivirus. PLoS One, 7(10): e47829.

White, S. N., Mousel, M. R., Reynolds, J. O., Herrmann-Hoesing, L. M., & Knowles, D. P. (2013). Deletion variant near ZNF389 is associated with control of ovine lentivirus in multiple sheep flocks. Animal Genetics, 45(2), 297-300.

White, S. N., Mousel, M. R., Reynolds, J. O., Lewis, G. S., & Herrmann-Hoesing, L. M. (2009). Common Promoter deletion is associated with 3.9-fold differential transcription of ovine CCR5 and reduced proviral level of ovine progressive pneumonia virus. Animal Genetics, 40(5), 583-589.

Yaman, Y., Keles, M., Aymaz, R., Sevim, S., Sezenler, T., Onaldi, T., Kaptan, C., Başkurt, A., Koncagül, S., Oner, Y., Oztürk, E, E., Iriadam, M., Un, C., & Heaton, M. P. (2019). Association of TMEM154 variants with visna/maedi virus infection in Turkish sheep. Small Ruminant Research, 177: 61–67.

Yavru, S., Şimşek, A., Bulut, O., & Kale, M. (2012). Konya bölgesi koyunlarında Maedi-Visna Virus enfeksiyonu üzerine serolojik araştırma. Eurasian Journal of Veterinary Sciences, 28(3), 142-148.

Livestock Studies, 2020; 60 (2): 74-80
http://doi.org/10.46897/livestockstudies.846420

REVIEW ARTICLE

LIVESTOCK STUDIES

The main aspects of andrological evaluation of bucks

Mehmet Borga Tırpan[1], Derya Şahin[2*], Fırat Korkmaz[2]

[1] Ankara University, Faculty of Veterinary Medicine, Department of Reproduction and Artificial Insemination, Ankara, Turkey
[2] International Center for Livestock Research and Training, Laboratory of Artificial Insemination, Ankara, Turkey

Article History

Received: 03 July 2020
Accepted: 19 November 2020
First Online: 25 December 2020

Corresponding Author

Tel.: +903128651196
E-mail: vh.sahinderya@outlook.com

Keywords

Andrological evaluation examination
Assisted reproductive technologies
Breeding soundness examination
Goat

Abstract

Goat breeding has economic importance specifically in developing countries in terms of fiber, milk, and meat production. To reach the increasing market demands, a thoroughly organized breeding program is required. While most breeders focus on the female part of reproduction management, male selection constitutes the overlooked half. However, this forgotten part should begin with male selection through an andrological evaluation examination (Breeding soundness exam (BSE)). After the male selection, semen collected from these bucks can be transported to other herds easily and genetic advance can be achieved in a shorter period with the help of assisted reproductive techniques. Using selected males' semen with artificial insemination (AI) in goats can bring huge improvement at goat breeding with its rapid and reform triggering effects. However, there are still missing pieces in terms of andrological examination evaluation and assisted reproductive techniques in goats and future research is still required to complete this puzzle. This review will focus on the selection of bucks and the advances in most commonly used assisted reproduction techniques in the field level.

Introduction

Goats have considerable economic importance and triggering impact in a wide scope of rural production systems (Fatet *et al.,* 2011), especially in developing countries. Their natural ability to use pastures and water efficiently and have a higher resistance to heat and cold stress allows it to be easily bred in a variety of climatic and geographical conditions (Gebre, 2007; Delgadillo *et al*., 1997). Additionally, its cultural role in some societies can not be ignored by explaining its spreading on the earth. In a temperate climate, they are mainly used for dairy, but they can also be used for meat and fiber production. In this zone, goats are seasonally polyestrous animals. The length of the sexual season varies with day length, latitude, climate, breed, nutrition, presence or absence of male and other unknown conditions. The main environmental factor affecting the sexual season of goats in temperate regions is the alterations in day length, which is called photoperiodism. Moreover, the largeness of serum gonadotropin and testosterone secretion of mature bucks in response to day length changes show breed differences. Additionally, does' follicular activity changes during the year in depending on the prolactin secretion and photoperiodism as similar bucks.

In bucks, puberty is associated with a marked increase in testosterone secretion, spermatogenesis and mating behavior. Ejaculation of viable sperm occurs at 4 to 6 months of age, and young bucks have 40 to 60% of their mature weights. Despite the age of sexual maturity linked to breed, it generally occurs 10-12 months old of age (Demir *et al.,* 2018; Hafez &Hafez, 2000; Chakraborty *et al.,* 1989). During the breeding season, does undergo several successive estrus cycles and the number of these cycles depends on the length of the breeding season and goat breed. In goats, the length of the estrus cycle is average 17 days but varying from 17 to 25 days. In this period, copulation usually occurs before ovulation, thus sperm are presented in the oviduct and also stored in the cervix, up to 3 days, and are incessantly released into the uterus, where they survive for

about 30 hours by this time. The embryo is implanted in the uterus 18 to 22 days after the onset of estrus and the length of gestation for the most breed of goats is an average of 144 days (Fatet *et al.,* 2011; Ridler *et al.,* 2012).

In goat breeding, determining the fertility characteristics of buck and doe has great importance for the reproductive success of the herd. As our focus, determining the fertility of bucks carries the success of fertilization in goat breeding with replacing the missing parts of reproduction for facilities.

Andrological Evaluation Examination

Although, numerous does are generally bred to a single buck, male fertility has a great importance in reproduction. Therefore, evaluation of male fertility has a priority to reach breeding success (Gebre, 2007; Chacon *et al.,* 1999). In various studies, reproduction in males determined mainly as affected by nutrition, genotype, season, management, and diseases (Dowsett & Knott, 1996; Bielli *et al.,*2002; Karagiannidis *et al.,* 2000). For optimal production rates in farms, bucks with high breeding abilities and genetic potential for rapid and efficient growth should be selected. These bucks can be selected by the utilization of an andrological evaluation examination protocol and can enhance the economical value of the herd (Pezzanite *et al.,* 2018). This protocol also helps prevent infertility problems from forming in a herd (Gebre, 2007; Goulet & Fthenakis, 2010). Andrological evaluation examination, also called breeding soundness examination (BSE), consists of a general physical examination, assessment of mating ability, and a genital tract examination of both the external and internal genitalia (including scrotal circumference measurement), and semen quality evaluation (Ridler *et al.,* 2012; Sathe & Shipley, 2014; Pezzanite *et al.,* 2018). Semen assessment which includes volume, motility, concentration, and morphology gives a general idea about breeding potential as well as freezability of the sample. Unlike bull andrological examination, buck andrological examination criteria are not yet fully developed or commonly used (Chacon *et al.,* 1999; Kennedy *et al.,* 2002). This evaluation should be done at least one month before the breeding season so that replacement can be possible before it is too late (Gouletsou & Fthenakis, 2010). However, it should not be forgot that the success of bucks of different ages and breeds undergoing andrological examination is variable.

Physical Examination

During the physical examination, mainly body condition score (BCS) and structural soundness are assessed under international or local breed criteria. In addition to these parameters, the history of the buck in terms of past diseases and breeding performance should be evaluated systemically. BCS is scaled from 1 to 5 and in terms of reproductive performance and efficacy, the optimal is known to be 3 to 3.5 (Pezzanite *et al.,* 2018; Menegassi *et al.,* 2014). It is also advised that the buck starts the reproductive season with a 3.5 to 4 BCS, as it will use a considerable amount of body weight during the breeding season (Gouletsou & Fthenakis, 2010). The age has also a crucial role for male fertility; optimal breeding age of bucks is considered to be 6 months to 4 years old (Sathe &Shipley, 2014). In terms of structural soundness feet and legs have the utmost importance. Along with the health of hooves; teeth and eyes are substantial parameters. Other diseases such as pneumonia, internal or external parasites, and especially brucellosis should also be checked (Pezzanite *et al.,* 2018). All breeder males should be also tested serologically for the following agents which are the main causes of infertility in males: *Actinobacillus seminis, A. actinomycetemcomitans, Histophilus ovis, Haemophilus* spp., *Corynebacterium pseudotuberculosis ovis, B. melitensis* and *Chlamidophila abortus* (Sathe &Shipley, 2014). Additionally, in order to protect the health of the herd and prevent possible aborts and orchitis, it is necessary to take precautions such as vaccination or the eliminating sick animals from the herd against viral infections (Border Disease (Pestivirus), Bluetongue (Orbivirus)) and parasitic infestations (Toxoplasmosis (*Toxoplasma gondii*)) that can be related by copulation (Menzies, 2012; Goulet & Fthenakis, 2015).

Examination of Reproductive Organs

This stage of andrological examination includes observation and palpation of penis, prepuce, scrotum, testis, and epididymis as well as epididymal measurements.

Anatomically, the male reproductive system consists of a pair of extra-abdominally located testes suspended in the scrotum, a fibroelastic penis, and accessory sex glands. The testes are already placed in the scrotum at the time of birth (Gier & Marion, 1970). Testes suspend vertically in the scrotum with the head of the epididymis located at the proximal end of the testes and its tail extending towards caudal poles. The testes of a buck weigh approximately 130-160 grams each. Scrotal circumference of a buck should be 28 cm and above, although it depends on various factors such as breed, age and body weight (Ridler *et al.,* 2012; Tibary, 2018). The penis has a sigmoid flexure located caudal to the spermatic cord and an urethral process is located to project 2-3 cm beyond the glans penis (Sathe & Shipley, 2014). Furthermore, bucks have all three accessory sex glands, vesicula seminalis being the largest. The prostate is disseminated and surrounds the wall of the pelvic urethra.

A pair of bulbourethral glands are situated caudal to the prostate and on the dorsal surface of the urethra (Haigh, 2007). All observable parts of this system should be normal and in harmony with other body systems.

The prepuce and penis should be both inspected visually and by palpation to make sure there are no signs of injury, adhesions, or other pathologies. Palpation of the accessory glands is not as easily done as in bulls; however, it can be done using one or two fingers or by transrectal ultrasonography (Tekin *et al.,*2019). Testicular traits are properties correlated with sperm production and animal fertility. Scrotal shape and content are parameters closely related to fertility parameters (Gebre, 2007; Sathe &Shipley, 2014; Coulter & Foote, 1977; Ott & Memon, 1980). During palpation of testicles and scrotum; the testes should be firm, movable within the scrotum, and similar in size. During this examination, epididymis should also be palpated in terms of any hardening or swelling as it can be an indicator of important diseases such as brucellosis. Scrotal circumference and testicular consistency are used to determine reproductive capacity because the scrotal circumference is a method of measuring testicular mass and when combined with testicular consistency it can be an indirect indicator of spermatogenetic capacity (Gebre, 2007; Ridler *et al*., 2012; Sathe &Shipley, 2014; Gouletsou & Fthenakis, 2010; Daudu, 1984, Pezzanite *et al.,* 2018; Filazi *et al.,* 2017) (Figure 1).

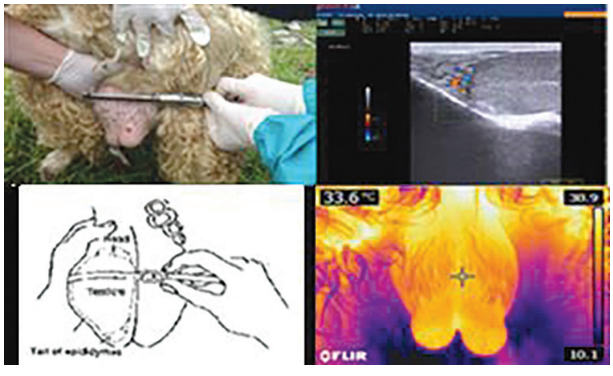

Figure 1: A: Testicular length measurement, B: Ultrasonographic Imaging, C: Circumference measurement, D: Testicular thermography imaging (Daskin *et al.*, 2015).

It was also shown that scrotal circumference is correlated to both age and body weight, with the latter being more significant (Sathe &Shipley, 2014; Pezzanite *et al.,* 2018). Recently additional procedures have been introduced in this part of the andrological evaluation. These procedures include ultrasonographic imaging of the testes (Sathe &Shipley, 2014; Gouletsou & Fthenakis, 2010). Up to date, ultrasonographic imaging has been used in bucks to determine testicular and epididymal measurements and testicular degeneration (Raji *et al.,*2016; Ahmad & Noakes, 1995)(Figure 1). Testicular echo-texture can be used to understand fertility. Healthy, normal buck testes are homogenously echogenic (Ahmad *et al.,*1991). The mineralization within the testicular parenchyma can be seen using ultrasonographic monitoring with dense hyperechoic areas and acoustic shadowing (Ahmad & Noakes, 1995). Another option was introduced to the examination of reproductive organs: Thermography. Even though no difference in terms of scrotal temperature was observed among bucks examined (Daskin *et al.,* 2015), this method may give insights about inflammation in the area (Figure 1). Thus, further research on this topic is required.

Semen Analyses

Semen collection and analyses form the last part of an andrological evaluation examination (Tirpan & Tekin, 2015). There are two methods generally used for collecting semen from the buck: using artificial vagina or electro-ejaculator (Sathe &Shipley, 2014). Recently, a novel semen collection method was also described: Transrectal digital massage. In this method, after the feces is removed from the rectum, the seminal vesicles and ampulla are located. On this part of the reproductive organs, back and forth vigorous motion is applied and is continued for up to 5 minutes and thus semen is collected (Tekin *et al.,*2017). It is the best time to collect semen is during the breeding season. For the artificial vagina method, the artificial vagina should consist of a hose 20-25 cm in length and 5 to 7 cm in diameter, with a rubber liner. It is critical to have the interlining lubricated, with the temperature of the artificial vagina approximately 39 ℃. As the buck mounts the doe, his penis is gently guided inside the artificial vagina. It is also important to an artificial vagina to have optimal pressure. The glass collecting tube warm also should be kept 37 ℃ to avoid cold shock until the semen evaluation. (Sathe &Shipley, 2014; Hafez &Hafez, 2000). In the other method, electroejaculation is usually preferred in bucks that are not trained for the artificial vagina. This is an easy to use method for the practitioner if the animal is well restrained or sedated, however, the semen sample obtained is not always in the optimal state. The electro ejaculator procedure is applied by putting a bipolar electrical probe into the buck's rectum. Low voltage electrical stimulation is given for 2 to 4 seconds at 10 to 20 seconds interim until ejaculation occurs. The sample collected using this method may be tainted with urine, may have a high volume but low concentration or higher concentrations of sodium and potassium (Sathe &Shipley, 2014). Semen volume varies considering the collection method. Fewer volumes are provided from artificial vagina comparing to electroejaculation collec-

tions. However, the electroejaculation method has several disadvantages as mentioned before.

Semen quality is mainly determined by the evaluation of spermatozoa motility, concentration, and morphology (Ott &Memon, 1980). In general, the minimal standards for the classification of buck semen samples are at least 85% motility, less than 10% morphological abnormalities, 6 to 10 x 10^8 sperm per ml, and at least 0.5 to 2 ml per ejaculate. A single test does not accurately predict the fertility of the sperm sample, but examining the various physical properties of the sperm can help determine the fertility potential (Kimberling, 1984; Hafez & Hafez, 2000; Tibary, 2018). In addition, semen evaluation grants a general idea of testicular and epididymal function, allowing the prevention of housing an infertile or subfertile animal (Gebre, 2007; Rodrigues-Martinez, 2003).

Beside all these classical methods, semen evaluations are mostly carried out by ancillary evaluation methods (computer-assisted semen analyse system and flow cytometry etc.) (Gürler *et al.,*2012).

Ancillary Semen Evaluation Methods

Various methods have been used to evaluate sperm ancillary. Some of them are electron microscopy, sperm chromatin structure assay (SCSA), flow cytometry, and computer-assisted semen analyses system (CASA). Flow cytometry and CASA are two methods that used commonly and enable sperm to be examined in many ways in detail (Hafez & Hafez, 2000, Korkmaz & Cil, 2020) (Figure 2).

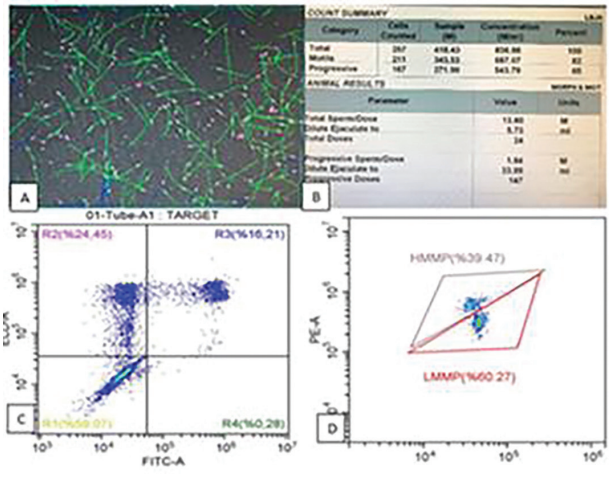

Figure 2: A-B: CASA morphometry and motility analysis, C: Flow cytometric PMAI (Plasma membrane and acrosome integrity) analysis, D: Flow cytometric MMP (Mitochondrial membrane potential) analysis.

Computer Assisted Semen Analyses (CASA)

CASA is a functional and useful method to reveal sperm populations and subpopulations in an ejaculate. Determination of motility and kinetic parameters of sperm in the ejaculate shows whether they are suitable for freezing and insemination (Tsakmakidis, 2010). These parameters obtained with CASA have been associated with fertility in many studies. The main parameters we can obtain regarding sperm populations with CASA are total motility, progressive motility, VAP (Average path velocity), VSL (Progressive velocity), VCL (Curvilinear velocity), BCF (Beat frequency), STR (Straightness), LIN (Linearity), ALH (Lateral head amplitude), etc. values. These parameters give us an idea of the sperm ability for fertility, and the power of advance in the female genital tract (Hirano *et al.,* 2001). When evaluating goat sperm in the CASA system, the point to be considered to obtain an objective result is the selection of specific settings for the buck. However, the values that obtain from the assessment from these systems should be evaluated with minimum and maximum limits for buck semen (Palacin *et al., 2013;* Tekin & Daşkin, 2016; Anand *et al.,* 2016).

Flow Cytometry

Flow cytometry is often used in andrology laboratories to use for sperm concentration, plasma membrane and acrosome integrity, apoptosis, mitochondrial membrane potential, the capacitation, oxidative stress, lipid peroxidation, DNA integrity analyzes (Korkmaz & Cil 2020). The purpose of these analyzes is to determine the quality of sperm like all other analyzes. Flow cytometry allows the objective, rapid and simultaneous analysis of several properties in a large number mass of spermatozoa, suggesting that the results of flow cytometric analysis may allow the estimation of the fertility of a semen sample. The results to be obtained from flow cytometry analysis make it easy to estimate the fertility of the semen sample with its reliable and rapid values (Peterson *et al*., 2007; Tsakmakidis, 2010).

Assisted Reproductive Techniques

Up-to-date, assisted reproductive techniques frequently used in goats include; synchronization of estrus and ovulation, estrus detection methods, and different insemination techniques. Controlling the reproduction of goats by inducing ovulation via hormonal treatments, manipulation of photoperiod, modulation of estrus cycles brings it about group kidding over a limited period (Sathe &Shipley, 2014). Goat reproduction management presents these main advantages; synchronizing pregnancies to form a kidding period in a selected season

and management of genetic resources (Sathe &Shipley, 2014).

Although all of the artificial insemination methods used in goats have their advantages and disadvantages, the methods used can be listed as follows: vaginal, cervical, transcervical, and laparoscopic, or intrauterine (Daskin *et al.,*2016). In goats, artificial insemination is usually performed using fresh semen because of the hardships faced during freezing or chilling semen (Chesh *et al.,* 2012). When processing goat semen Tris-egg yolk-based extenders are most commonly using agents. Since semen cryopreservation is a valuable technique for goat production allowing semen to be stored for later use (Konyalı *et al.,* 2013), researchers are still trying (1) different extenders (Roof *et al.,* Küçük et al., 2014; Yodmingkwan *et al.,* 2016), (2) freezing protocols (Küçük et al., 2014; Salmon *et al.,* 2017; Inanç *et al.,* 2017) and (3) additives (Konyalı *et al.,* 2013; Salmon *et al.,* 2017) to increase cryo survival of goat semen. If the doe is synchronized; the optimal timing for insemination, including laparoscopic procedure, is usually around 45 hours after progestagen device removal (Cseh *et al.,* 2012). Artificial insemination in goats results in acceptable pregnancy rates when fresh semen is used for vaginal or intra-cervical insemination and frozen semen is used for trans-cervical intrauterine or laparoscopic insemination. Insemination with fresh semen results in pregnancy rates close to natural mating while cervical insemination with frozen semen does not yield satisfactory results (Tırpan *et al.,* 2017; Tırpan *et al.,*2019). With the laparoscopic insemination technique recently developed, better and more consistent pregnancy rates may be achieved even with frozen semen. Cervical insemination is the most preferred method in goats (Cseh *et al.,* 2012).

Conclusions

In conclusion, the usage of the andrological examination technique carries the utmost importance in the selection of breeder bucks. The common herd reproductive systems have been used in goat breeding is uncontrolled natural breeding. In such systems, bucks and does are kept together in the same environment regardless of their reproductive characteristics. One step further, in herds where limited controlled natural mating is applied, goats are selected as breeders according to their phenotypic features. However, a little attention is given to the fertility characteristics of the female, and the male andrological and spermatological evaluation can bring success. With enhancing technology; ultrasonography, thermography, computer-assisted methods, and flow cytometry devices finding their place among andrological examinations aid in the better determination of bucks' breeder values. By means of these technologies integrating with andrological examination systematics, parameters such as genetic advance, determination of hereditary and contagious diseases, litter size, estimation of breeding value can be determined more healthily and contribute to the breeding of animals of economic value. The semen collected from bucks of which breeding values are revealed by innovative techniques can be used to inseminate females and reaching genetically superior herds is simplified. With this vision in mind, future research in determining easily applicable technologies and forming a species-specific database should be pursued.

References

Ahmad, N., & Noakes, D. E. (1995). Ultrasound imaging in determining the presence of testicular degeneration in two male goats. *British Veterinary Journal*, 151: p.101-110.

Ahmad, N., Noakes, D. E., & Subandrıo, A. L. (1991). B-mode real time ultrasonographic imaging of the testis and epididymis of sheep and goats. *Veterinary Record*, 128: p.491-496.

Anand, M., Yadav, S., Kumar, J., & Madan, A. K. (2016). Assessment of motion characteristics and path velocities exhibited by barbari buck spermatozoa. *Veterinary Practitioner*, *17*(2), 263–264.

Bielli, A., Pérez, R., Pedrana, G., Milton, J., López, A., Blackberry, M. A., Duncombe, G., Rodriguez-Martinez, H., & Martin, G. B. (2002). Low maternal nutrition during pregnancy reduces the number of sertoli cells in the newborn lamb. *Reproduction Fertility Development*, 14: p.333-337.

Chacon, J., Perez, E., Muller, E., Söderquist, L., & Rodriguez-Martinez, H. (1999). Breeding soundness evaluation of extensively managed bulls in Costa Rica. *Theriogenology*, 52: p.221-231.

Chakraborty, P. K., Stuart L. D., & Brown J. L. (1989). Puberty in the male Nubian goat: serum concentrations of LH, FSH and testosterone from birth through puberty and semen characteristics at sexual maturity. *Animal Reproduction Science*, 20:91.

Coulter, G. H., & Foote, R. H. (1977). Relationship of body weight to testicular size and consistency in growing Holstein bulls. *Journal of Animal Science*, 44: p.1076-1079.

Cseh, S., Faigl, V., & Amiridis, G. S. (2012). Semen provessing and artificial insemination in health management of small ruminants. *Animal Reproduction Science*, 130: p.187-192.

Daskin, A., Stelletta, C., Pascale, L., Oztutar F., Alemdar, H., Turri, F., Tırpan, M. B., Vencato, J., Tekin, K., & Pizzi, F. (2015). "Integrated Andrological Evaluation in Angora Goat." *Animal Reproduction*, *12*(3): 792.

Daskin, A., Tekin, K., Tırpan, M. B., Inanç, M. E., Çil, B., & Alemdar, H. (2016). The effect of different insemination techniques and cervical conformation index on fertility rates in Angora goat. *Animal Reproduction Science, 100*(169), 116.

Daudu, C. S. (1984). Spermatozoa output, testicular sperm reserve and epididymal storage capacity of the Red Sokoto goats indigenous to northern Nigeria. *Theriogenology*, 21: p.317-324.

Delgadillo, J. A., Malpaux, B., & Chemineau, P. (1997). La reproduction des caprins dans les zones tropicales et subtropicales, INRA, *Productions Animales*, *10*(1): p.33–41.

Demir, H., Elmaz, Ö., & Cerit, H. (2018). Keçilerde puberta ve eşeysel olgunluk yaşının belirlenmesi ile ilgili araştırmalar. *Uludağ Üniversitesi Veteriner Fakültesi Dergisi*, (January 2019).

https://doi.org/10.30782/uluvfd.415214.

Dowsett, K. F. & Knott, L. M. (1996). The influence of age and breed on stallion semen. *Theriogenology*, 46: p.397-412.

Fatet, A., Pellicer-Rubio, M. T., & Leboeuf, B. (2011). Reproductive cycle of goats. *Animal Reproduction Science*, 124: p.211-219.

Filazi, A., Yurdakök, B., Tekin K., Tırpan, M. B., & Daşkın, A. (2017). In vitro toxicity of some pesticides on goat and dog spermatozoa. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi*, 23(2).

Gebre, Y. M. (2007). Reproductive traits in Ethiopian male goats with special reference to breed and nutrition. Doctoral thesis, Swedish University of Agricultural Sciences, Uppsala.

Gier, H. T. & Marion, G. B. (1970). The Testis Development, Anatomy and Physiology, Volume I, Academic Press, New York and London.

Gouletsou, P. G., & Fthenakis, G. C. (2010). Clinical evaluation of reproductive ability of rams. *Small Ruminant Research*, 92: p.45-51.

Gouletsou, P. G., & Fthenakis, G. C. (2015). Microbial diseases of the genital system of rams or bucks. *Veterinary Microbiology,* 181: 130-135.

Gürler, H., Siuda, M., Göbel, H., Tırpan, B., Leiding, C., & Bollwein, H. (2012). Effects of a prolongation of equilibration time up to 96 hours on quality of cryopreserved bovine sperm. *Reproduction in Domestic Animals*, 47(Supplement 2): p.25.

Hafez, E. S. E., & Hafez, B., (2000). Reproduction in Farm Animals. 7th Edition. ISBN 0-683-30577-8.

Haigh, J. C. (2007). Current Therapy in Large Animal Theriogenology, Second Edition. Saunders Elsevier, Missouri.

Hirano, Y., Shibahara, H., Obara, H., Suzuki, T., Takamizawa, S., Yamaguchi, C., & Sato, I. (2001). ANDROLOGY: Relationships Between Sperm Motility Characteristics Assessed by the Computer-Aided Sperm Analysis (CASA) and Fertilization Rates In Vitro. *Journal of Assisted Reproduction and Genetics*, *18*(4), 215–220. https://doi.org/10.1023/A:1009420432234.

Inanç, M. E., Tekin, K., Olğaç, K. T., Tırpan, M. B., Alemdar, H., Çil, B., Kaya, U., Stelletta, C., & Daşkın, A. (2017). Effects of region and invidualism traits on sperm freezeability of Angora Goats. *Journal of Turkish Veterinary Medical Society*, 88, 31-39.

Karagiannidis, A., Varsakeli, S., & Karatzas, G. (2000). Characteristics and seasonal variations in the semen of Alpine, Saanen and Damascus goat bucks born and raised in Greece. *Theriogenology*, 53: p.1285-1293.

Kennedy, S. P., Spitzer, J. C., Hopkins, F. M., Higdon, H. L., & Bridges, W. C. (2002). Breeding soundness evaluations of 3648 yearling beef bulls using the 1993 society for Theriogenology guidelines. *Theriogenology*, 58: p.947-961.

Kimberling, C. V. (1984). Breeding Soundness Evaluation of Rams. *Journal of American Veterinary Medical Association,* 185: 325-325.

Konyalı, C., Tomas, C., Blanch, E., Gomez, E. A., Graham, J. K., & Moce, E. (2013). Optimizing conditions for treating goat semen with cholesterol-loaded cyclodextrins prior to freezing to improve cryosurvival. *Cryobiology*, 67: p.124-131.

Küçük, N., Aksoy, M., Uçan, U., Ahmad, E., Naseer, Z., Ceylan, A., & Serin, İ. (2014). Comparison of two different cryopreservation protocols for freezing goat semen. *Cryobiology*, 68: p.327-331.

Menegassi, S. R. O., Barcellos, J. O. J., Borges, J. B. S., Canozzi, M. E. A., Perpolli, V., Junior, C. K., Lopes, F. G., & Cervo, H. J. (2014). Breeding soundness examination: Understanding the causes of examination failure in young and mature rams. *International Journal of Plant and Animal Sciences*, 2(2): p.98-104.

Menzies, P. I. (2012). Vaccination programs for reproductive disorders of small ruminants. Anim. Reprod. Sci. 130, 162–172.

Ott, R. S., & Memon, M. A. (1980). Breeding soundness examinations of rams and bucks, a review, *Theriogenology*, *13*(2): p.155-164.

Palacín, I., Vicente-Fiel, S., Santolaria, P., & Yániz, J. L. (2013). Standardization of CASA sperm motility assessment in the ram. *Small Ruminant Research, 112*(1–3), 128–135. https://doi.org/10.1016/j.smallrumres.2012.12.014

Peterson, K., Kappen, M. A. P. M., Ursem, P. J. F., Nöthling, J. O., Colenbrander, B., & Gadella, B. M. (2007). Microscopic and flow cytometric semen assessment of Dutch AI-bucks: Effect of semen processing procedures and their correlation to fertility. *Theriogenology, 67*(4), 863–871. https://doi.org/10.1016/j.theriogenology.2006.11.003.

Pezzanite, L., Bridges, A., Neary, M., & Hutchens, T. (2018). Breeding soundness examinations of rams and bucks, Purdue Extension. *Animal Sciences* [cited 2018 26 February]; Available from: https://www.extension.purdue.edu/extmedia/AS/AS-599-W.pdf.

Raji, L. O., Ajala, O. O., & Ameen, S. A. (2016). Testicular ultrasound as a breeding soundness examination and biometric tool for West african dwarf buck goats. *Slovak Journal of Animal Science*, (1): p.8-16.

Ridler, A. L., Smith, S. L., & West D. M. (2012). Ram and buck management. *Animal Repdoruction Science*, 130: p.180-183.

Rodriguez-Martinez, H. (2003). Laboratory semen assessment and prediction of fertility: still utopia? *Reproduction in Domestic Animals*, 38: p.312-318.

Roof, D. J., Bowley, S., Price, L. L., & Matsas, D. J. (2012). Comparison of two commercial extenders for cryopreservation of goat semen without sperm washing. *Theriogenology*, 77: p.412-420.

Salmon, V. M., Lerlerc, P., & Bailey, J. L. (2017). Novel technical strategies to optimize cryopreservation of goat semen using cholesterol-loaded cyclodextrin. *Cryobiology*, 74: p.19-24.

Sathe, S., & Shipley, C. (2014). Animal Andrology: Theories and Applications. Wagga Wagga, Australia.

Tekin, K., & Daşkin, A. (2016). Effect of different extenders on motility and some sperm kinematics parameters in Norduz goat semen. *Turkish Journal of Veterinary and Animal Sciences*, 40(4), 490–495. https://doi.org/10.3906/vet-1509-5

Tekin, K., Çil, B., Alemdar, H., Olğaç, K. T., Tırpan, M. B., Daskin, A., & Stellatta, C. (2019). Semen collection by trans-rectal digital stimulation and insemination campaign in goat. *Andrologia, 52*(2), e13458. https://doi.org/10.1111/and.13458.

Tekin, K., Stelletta, C., Çil, B., Tırpan, M. B., Olğaç, K. T., Alemdar, H., & Daşkın, A. (2017). Trans-rectal semen collection and artificial insemination in Angora goat. *Reproduction in Domestic Animals, 52*(3): 64.

Tırpan, M. B., & Tekin, N. (2015). Effects of boron (sodium pentaborate), added instead of Tris components, on freezing and post-thaw quality of Angora buck semen. *Ankara Üniversitesi Veteriner Fakültesi Dergisi*, 62, 295-302.

Tırpan, M. B., Tekin, K., Çil, B., Alemdar, H., İnanç, M. E., Olğaç, K. T., Stelletta, C., & Daşkın, A. (2019). The effects of different PMSG doses on estrus behavior and pregnancy rate in Angora goats. Animal, *13*(3), 564-569., Doi: 10.1017/S1751731118001908.

Tırpan, M. B., Tekin, K., Stelletta, C., & Daşkın, A. (2017). Intracervical artificial insemination of Saanen goats in different breeding age with frozen- thawed semen. 21th Annual Conference of the European Society for Domestic Animal Reproduction, 52(3), 138-138.

Tibary, A., Boukhliq R., El Allali K. (2018). Ram and Buck Breeding Soundness Examination. Revue Marocaine des Sciences Agronomiques et Vétérinaires. 6: 241-255.

Tsakmakidis, I. A. (2010). Ram semen evaluation: Development and efficiency of modern techniques. *Small Ruminant Research, 92*(1–3), 126–130. https://doi.org/10.1016/j.smallrumres.2010.04.017

Yodmingkwan, P., Guntaprom, S., Jaksamrit, J., & Lertchunhakiat, K. (2016). Effects of extenders on fresh and freezing semen of boer goat. *Agriculture and Agricultural Science Procedia*, 11: p.125-130.