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Research article

Survival analysis and reproductive performance of Dorper x Tumele sheep

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ARTICLE INFO	A B S T R A C T			
Keywords: Crossbreeding Diseases Fitness traits Mothering ability Risk factors	Productivity and profitability of sheep farming are highly influenced by lamb survival and ewe reproductive performance. Thus, this study was conducted to evaluate the survival and reproductive performance of crossbred sheep. Data collected from 2009 to 2018 from Sirinka sheep breeding stations were utilized for this study. Survival analysis was conducted by using Survival Kit 6.12 software with the Weibull model and the general linear model of SAS 9.0 was used to analyze reproductive traits. The overall mean survival rate of Dorper x Tumele crossbred lambs at 3, 6 and 12 months of age were 86.0, 76.6, and 67.9%, respectively. About 46.8% of mortality from the total death was observed during the first 120 days of life. Gastrointestinal parasites, pneumonia and septicemia were the major causes of lamb mortality. Birth weight, birth type, sex and year of lambing were the most important risk factors for survival of crossbred lambs. The overall least-squares means for litter size at birth, litter size at weaning, total litter weight at birth and total litter weight at weaning were 1.10 lambs, 0.94 lambs, 3.28 kg and 155 kg, respectively. Birth type, sex and year of lambing were the most determinants of ewe pro-			

3.28 kg and 15.5 kg, respectively. Birth type, sex and year of lambing were the most determinants of ewe productive traits. Tumele and their crossbred sheep had good mothering ability necessary to successfully raise lambs to weaning. The current crossbreeding program which aims to improving growth performance had a positive influence on the survival rate of lambs. Improvement of environmental in the flock, special care for small lambs and indirect selection based on birth weight would lead to further survival improvement.

1. Introduction

Sheep production is a major component of the livestock sector in Ethiopia. There are around nine sheep breeds (Gizaw et al., 2008) and the total population is about 29.7 million (CSA, 2016). However, the productivity of indigenous sheep breeds and human population growth is unbalanced. Thus, to meet the ever-increasing demand for animal products and thus contribute to economic growth, intensification of sheep production using more productive exotic genotypes has been advocated as a means of improving the livelihoods of farmers. Accordingly, Romney, Corriedale, Hampshire, Rambouillet, and Awassi sheep breeds were imported to Ethiopia in different years since 1944 (Getachew et al., 2016). However, the contribution of these breeds except Awassi sheep was negligible. Consequentially, the project entitled Ethiopian Sheep and Goat Productivity Improvement Program (ESGPIP) launched crossbreeding of indigenous ewes with Dorper sire breed in

different parts of the country with a goal of improvement of meat production since 2007.

Despite their productivity, poor fitness of the crossbreed sheep is a great problem faced by sheep farming in tropics. Flock productivity and profitability of sheep farming are highly influenced by lamb survival and reproductive performance. The increase in the number of reared lambs per maintained ewes can be considered as the increase of fertility, lambing, number of lambs at birth and lamb weaning (Yavarifard et al., 2015). Currently, crossbred rams were selected and shared to serve the ewes in the communities. However, in order to further scale out this crossbreeding program, it is important to evaluate the fitness traits (survival and reproductive performances) of the crossbreds. Moreover, it is paramount to have information about the influences of genetic and non-genetic factors on fitness traits. However, there is little evidence in this regard in Ethiopia. Therefore, the objectives of this study were to evaluate the survival and reproductive performance of crossbred sheep.

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2. Materials and methods

2.1. Study area and flock management

The study was conducted at Sirinka sheep breeding station which is located 508 km away from Addis Ababa at an altitude of 1850 m.a.s.l and at 11°45′ 00″ N and 39°36′ 36″ E. The mean annual rainfall amount of the area is on average about 950 mm. The area is a moderately warm temperature zone with a mean daily temperature ranges from 13.7 - 26.4 $^{\circ}$ C.

Sheep were allowed to lambing throughout the year following a natural controlled breeding program. The ewes were mated with rams at a ratio of 20–30 to 1 and the sire kept with ewes for 45 days. At mating, ewes were herded with their respective sire groups during the daytime and depart for the night time.

Newly born lambs were kept together for up to three to five days with their dam then after lambs were isolated and suckled three times per day until three months (weaning) age. In addition to their dam's milk, lambs had access to concentrate feed (100 g/day/lamb) until weaning. All breeding sheep were allowed graze/browse on natural pasture for 6:00 h from 9:00-11:00 h in the morning and from 14:00-16:00 h in the afternoon. Both weaned male and female lambs were supplemented with 200 g/day/animal concentrate mix once in the afternoon. During late gestation and early lactation period, 400 g of concentrate mixture was provided in the evening hours after grazing/browsing per day per animal. However, the supplementation of the flock was not-regular due to financial limitation, i.e. during this period supplementation was allowed once in two days. All sheep were housed in semi-opened concrete barns at night and had access to water freely. Sheep were vaccinated against Sheep and Goat pox, Anthrax and Pestis Des Petites Ruminants diseases. They were treated regularly for internal and external parasites. And also dipped and sprayed for ticks, mites and other ectoparasite control and prevention.

2.2. Studied traits

The mortality, out flow and causes of the death or exit of the lambs from the flock up to yearling age, were recorded by veterinary experts and utilized in this study. The survival traits included in this a study were survival from birth to 3, 6 and 12 months of age. For each time of survival, a lamb attained a censored code for Weibull proportional hazards model (0 for right-censored and 1 for death related to viability). The total numbers of records were 530; the proportion of the right-censored records at 3, 6 and 12 months of age was 86.04, 76.60 and 67.92%, respectively. Lambs removed from the flock due to reasons not related to their viability *i.e.* disseminated to farmers for mating, died due to car accidents and culling related to flock management were not considered in this study.

The reproductive traits include litter size at birth (LSB), litter size at weaning (LSW), total litter weight at birth (TLBW) and total litter weight at weaning (TLWW). Litter size at birth is the number of lambs born alive per ewe lambing. Litter size at weaning is the number of lambs present at three months per ewe lambing, while their total weight signifies the litter weight at birth and three months per ewe lambing.

2.3. Statistical analysis

2.3.1. Survival analysis

The survival analysis was conducted using Survival kit version 6.12 software (Mészáros et al., 2013). The Weibull distribution assumptions for survival data at different ages were tested according to Ducrocq et al. (2000) by plotting the value of log [$-\log S(t)$] against log (t). Estimation of the survivor function was computed as follow (Kaplan and Meier, 1958):

 $S_{KM}\left(t\right)=\prod_{i:\ ti\ \leq\ t}\left[(n_{i}\text{-}d_{i})/n_{i}\right]$

Where, $S_{KM}(t)$ is the value of survival function at a time t_i , n_i is the number of lambs alive at time t_i and d_i is the number of lambs died at time t_i .

Then Weibull proportional hazard model for the death of a particular lamb at a time (t) was designed as follow:

$$\lambda(t) = \lambda_0(t) \ x \ exp \ [BT_i + SS_j + SX_k + DG_l + T_m + L_n + W_o]$$

where, $\lambda(t) =$ the risk of death or probability of lamb being died at time t, $\lambda_0(t) =$ the baseline hazard function with shape parameter p and scale parameter λ of the Weibull distribution or $\lambda_0(t) = \lambda \rho(\lambda t)^{p-1}$, BT_i is fixed effect of the ith birth type, SS_j is fixed effect of the jth season of birth, SX_k is fixed effect of the kth sex of lamb, DG_l is fixed effect of the lth dam genotype, T_m is fixed effect of the mth year of birth, L_n is fixed effect of the nth lamb blood level, and W_o is fixed effect of oth birth weight category. The importance of the explanatory variables was tested by using a likelihood ratio test to find out the best model.

2.3.2. Reproductive traits

The reproductive traits were analyzed by using a general linear model (GLM) procedure of SAS (2002). Differences between the least-square means of a trait for different genetic and non-genetic factors were tested using the Tukey-Kramer test based on the ANOVA result. The statistical models were as follow:

$$Y_{ijklm} = \mu + D_i + S_j + X_k + W_l + e_{ijklm}$$

where,

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Y<sub>ijklmn</sub> = the response variable
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\mu = overall mean
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 D_i = effect of ith genotype (2 levels: Tumele and Dorper x Tumele or F1)

 $S_i = effect \text{ of } j^{th} \text{ season of lambing (3 levels: main rain, short rain and dry)}$

 $X_k = effect \text{ of } k^{th} \text{ sex of lamb (2 levels: male and female)}$

 $W_1 = effect \text{ of } l^{th} \text{ year of lambing (10 levels: 2009–2018)}$

 $e_{ijklm} =$ random error term associated with each observation

3. Results and discussion

3.1. Survival and risk factors

The risk ratios and the influences of different risk factors are presented in Table 1. The overall mean survival rate of Dorper x local crossbred lambs at 3, 6 and 12 months of age was 86.0, 76.6, and 67.9%, respectively. Relatively higher mortality up to weaning (16.7%) and up to yearling age (36.3%) than the current finding was reported by Getachew et al. (2015) for Menz sheep. Likewise, the survival rate of crossbreds up to 3 months of age was higher than the value (81%) reported by Abebe et al. (2015) for Dorper x Menz crossbred sheep. However, relatively better survival (80%) at 6 months of age was noted by the same author. Based on the likelihood ratio test, birth weight, birth type, sex and year of lambing had a considerable (P < 0.05) influence of lamb survival. However, the season of lambing, dam genotype and blood level were not significant sources of variation for the survival of lambs at different ages.

The effect of birth type was higher in first the period and then it was declining over the period. The risk of death with twin born lambs was increased by 38% and 30% compared with single born lambs at 3 and 6 months of age, respectively. However, the influence of birth type at yearling age was found to be non-significant. These results are in agreement with previous studies (Casellas et al., 2007; Sawalha et al., 2007; Bangar et al., 2016; Abdelqader et al., 2017). According to Abdelqader et al. (2017), lambs born from twin or triplets litters were under very high risk to die from hypothermia, starvation, gastrointestinal

Table 1. Risk ratios for the explanatory variables.

P-value 1 * 0.0351 2 ** 0.1802 2 - 0.0072 4 0.3629 1 ns	NF 98 26 24 46 44 10 70	RR 1.00 1.30 1.14 1.00 0.53 0.67 1.00	P-value * - 0.0310 * 0.6332 - 0.0080 0.3046 *	NF 138 32 31 61 66 12	RR 1.00 0.91 2.34 2.01 1.00 0.92	P-value ns - 0.7097 ** 0.0019 0.0011 - 0.8035
* 0.0351 ** 0.1802 0.0072 0.3629 ns	98 26 24 46 44 10 70	1.00 1.30 1.14 1.00 0.53 0.67	* - 0.0310 * 0.6332 - 0.0080 0.3046 *	138 32 31 61 66 12	1.00 0.91 2.34 2.01 1.00 0.92	ns - 0.7097 ** 0.0019 0.0011 - 0.8035
- 60.0351 2 *** *** 0.1802 2 2 ** - 60.0072 6 ** 0.3629 7 ** ns	98 26 24 46 44 10 70	1.00 1.30 1.14 1.00 0.53 0.67	- 0.0310 * 0.6332 - 0.0080 0.3046 *	138 32 31 61 66 12	1.00 0.91 2.34 2.01 1.00 0.92	- 0.7097 ** 0.0019 0.0011 - 0.8035
0.0351 : ** 0.1802 : 0.0072 : 0.3629 : ns	26 24 46 44 10 70	1.30 1.14 1.00 0.53 0.67	0.0310 * 0.6332 - 0.0080 0.3046 *	32 31 61 66 12	0.91 2.34 2.01 1.00 0.92	0.7097 ** 0.0019 0.0011 - 0.8035
** 0.1802 : - • 0.0072 • 0.3629 : ns	24 46 44 10 70	1.14 1.00 0.53 0.67	* 0.6332 - 0.0080 0.3046 *	31 61 66 12	2.34 2.01 1.00 0.92	** 0.0019 0.0011 - 0.8035
0.1802 2 - 4 0.0072 4 0.3629 5 ns	24 46 44 10 70	1.14 1.00 0.53 0.67	0.6332 - 0.0080 0.3046 *	31 61 66 12	2.34 2.01 1.00 0.92	0.0019 0.0011 - 0.8035
	46 44 10 70	1.00 0.53 0.67	- 0.0080 0.3046 *	61 66 12	2.01 1.00 0.92	0.0011 - 0.8035
0.0072 • 0.3629 : ns	44 10 70	0.53 0.67	0.0080 0.3046 *	66 12	1.00 0.92	- 0.8035
0.3629 : ns	10 70	0.67	0.3046 *	12	0.92	0.8035
ns	70	1.00	*			
	70	1.00				*
		1.00		89	1.00	
0.0658	54	0.62	0.0141	81	0.69	0.0397
***			***			***
0.4024	10	0.19	0.0016	13	0.31	0.0172
0.0377	5	0.09	0.0003	8	0.20	0.0065
0.1148	5	0.14	0.0028	12	0.32	0.0461
0.8672	12	0.24	0.0036	15	0.39	0.0495
0.4901	9	0.66	0.4049	13	1.31	0.5822
0.1948	22	1.00	-	23	2.11	0.1739
0.7421	17	0.45	0.0815	18	0.65	0.2156
- :	18	0.36	0.0435	35	1.00	-
0.7248	21	0.48	0.0658	27	1.13	0.7641
	5	0.17	0.0112	6	0.30	0.0461
	0.1948 0.7421 - 0.7248 0.1262	0.1948 22 0.7421 17 - 18 0.7248 21 0.1262 5	0.1948 22 1.00 0.7421 17 0.45 - 18 0.36 0.7248 21 0.48 0.1262 5 0.17	0.1948 22 1.00 - 0.7421 17 0.45 0.0815 - 18 0.36 0.0435 0.7248 21 0.48 0.0658 0.1262 5 0.17 0.0112	0.1948 22 1.00 - 23 0.7421 17 0.45 0.0815 18 - 18 0.36 0.0435 35 0.7248 21 0.48 0.0658 27 0.1262 5 0.17 0.0112 6	0.1948 22 1.00 - 23 2.11 0.7421 17 0.45 0.0815 18 0.65 - 18 0.36 0.0435 35 1.00 0.7248 21 0.48 0.0658 27 1.13 0.1262 5 0.17 0.0112 6 0.30

infections and respiratory infections. Besides, lower birth weights, larger surface area to lose body heat, smaller reserves of body fat and competition with its litter mate for colostrum and milk are the other possible causes of poor survival of twins (Hatcher et al., 2009, 2010. This suggests that including litter size as a breeding goal trait increases the number of lambs born over time while decreasing their chances of survival. Besides, ewes that gave high birth weight in their lambs also had higher survival of lambs indicating the possibility of genetic improvement through selection using a maternal line.

The risk of mortality tended to decreases with the increases in the birth weight of lambs. The lower birth weight (<2.0 kg) was associated with a 62% increase in the risk of death at 3 months and a 14% increase in the risk of death at 6 months of age compared with lambs in the $2^{\mbox{nd}}$ birth weight category (2.1–2.9 kg). At 12 months of age, lambs in the 1st birth weight category (\leq 2.0 kg) and lambs in the 2nd birth weight category (2.1-2.9 kg) were associated with 134% and 101% increase in the risk of mortality, respectively when compared with 3rd birth weight category (3.0-3.9 kg). Likewise, Abdelgader et al. (2017) noted that small size lambs (<2 kg birth weight) were more susceptible to die than lambs born with greater birth weights (3-4 kg). High risk of mortality for lower birth weights can be related to reducing fetal lipid reserves (Casellas et al., 2007) which increases susceptibility to hypothermia and starvation and thereby attenuated lamb vitality. This suggests that manipulating ewe nutrition during pregnancy to increase birth weight will improve lamb survival.

The risk of death with female lambs was reduced by 38% and 31% compared with male lambs at 6 and 12 months of age, respectively. The superiority of female lambs in their survival was reported elsewhere (Sawalha et al., 2007; Barazandeh et al., 2012; Binabaj et al., 2013; Getachew et al., 2015; Bangar et al., 2016; Abdelqader et al., 2017). The exact causes of the superiority of female lambs in terms of survival rate were not noted in most of the studies. However, Dwyer (2003) found out that male lambs were slower to stand and suck than female lambs in Suffolk sheep breed. This could be the possible reason for the observed superiority in survival for females over males.

The year of lambing exerted a significant influence on the survival of crossbred lambs. The year 2010 was associated with an 84% decrease in the risk of mortality and the year 2014 was associated with a 176% increase in the risk of mortality at 3 months of age compared with lambs born in 2016. Likewise, at yearling age, the risk of death for lambs born during 2014 was increased by 111%, but lambs born in 2010 and 2018 reduced by 80% and 70%, respectively compared with lambs born in 2016. The influence of the year can be associated with variation in climatic conditions which affect lamb survival through the effects on the nutritional status of the grazing ewe and lambs.

The survival rate of lambs was decreasing at an increasing rate up to 120 days of age and decreasing at a decreasing rate afterward. About 46.8% of mortality from the total death was observed during the first 120 days of life (Figure 1). It is quite clear that lamb survival up to this age is directly or indirectly associated with the condition of ewes. Thus, there should be due attention (improving management) for both lambs and ewes in order to reduce the loss of lambs.

Gastrointestinal parasites, pneumonia and septicemia were the major causes of lamb mortality (Table 2). The grazing behavior of crossbreds, lack of appropriate post-lambing management and level of the fat reserve could be the possible reasons for the occurrence of internal parasites and pneumonia, respectively.

3.2. Reproductive performance and the influence of environmental factors

A reproductive trait of Tumele and their crossbred with Dorper sheep are shown in Table 3. The coefficient of variation for LSW and TLWW indicates that there is a variation among ewes in terms of raising lambs. The litter size at birth and at weaning for both genotypes in this study is comparable with the report of Mokhtari et al. (2010) for Kermani sheep and Taye et al. (2011) for Washera sheep. Except for TLWW, the reproductive performance of Tumele and their crossbred with Dorper sheep was found to be similar (P > 0.05). The superiority of crossbred ewes for TLWW could be due to the advantage of a non-additive genetic effect, as the heterosis for the reproductive trait is high. However, the observed





Table 2. Distribution of dead lambs up to yearling age into each cause of death category.

Causes of mortality	Ν	Mean \pm SE
Gastrointestinal parasites	59	34.2 ± 3.60
Pneumonia	30	17.4 ± 2.74
Septicemia	28	16.3 ± 2.66
Dermatitis	23	13.5 ± 2.45
Nervous disturbance	10	5.86 ± 1.68
Metabolic diseases	9	5.24 ± 1.60
Abscess	3	1.74 ± 0.94
Ecto-parasites	3	1.74 ± 0.94
Wound	3	1.74 ± 0.94
Conjunctivitis	2	1.16 ± 0.77
N number of dead lambs		

Table 3. Reproductive performance (LSM±SE).

Source of variation	Ν	LSB(lamb) LSM±SE	Ν	LSW(lamb) LSM±SE	Ν	TLBW(kg)	Ν	TLWW (kg)	
						LSM±SE		LSM±SE	
Overall	490	1.10 ± 0.01	483	0.94 ± 0.02	489	3.28 ± 0.04	415	15.5 ± 0.23	
CV (%)	490	4.06	483	39.2	489	22.5	415	26.3	
Genotype		ns		ns		ns		**	
Tumele	377	1.09 ± 0.01	370	0.95 ± 0.02	376	3.26 ± 004	324	15.1 ± 0.23	
D x T (F1)	113	1.11 ± 0.03	113	$\textbf{0.93} \pm \textbf{0.04}$	113	3.37 ± 0.09	91	16.4 ± 0.68	
Season		ns		ns		***		ns	
Dry	155	1.12 ± 0.02	156	$\textbf{0.99} \pm \textbf{0.03}$	155	3.27 ± 0.07^{b}	137	16.1 ± 0.46	
Main rain	105	1.08 ± 0.02	104	$\textbf{0.90} \pm \textbf{0.04}$	105	2.86 ± 0.07^{c}	84	14.5 ± 0.48	
Short rain	229	1.09 ± 0.02	223	$\textbf{0.93} \pm \textbf{0.03}$	229	$\textbf{3.48}\pm0.04^{a}$	194	15.6 ± 0.30	
Sex		ns		ns		*		**	
Female	244	1.11 ± 0.02	241	0.97 ± 0.02	244	3.21 ± 0.06	211	14.9 ± 0.27	
Male	246	1.08 ± 0.01	242	0.92 ± 0.02	245	3.36 ± 0.06	204	16.1 ± 0.38	
Year		ns		**		***		***	
2009	69	1.04 ± 0.02	69	0.94 ± 0.04^{bc}	69	3.23 ± 0.07^{cde}	65	16.0 ± 0.45^{bc}	
2010	61	1.09 ± 0.04	61	1.03 ± 0.04^{ab}	61	3.67 ± 0.14^{ab}	59	$16.1\pm0.58^{\rm bc}$	
2011	58	1.01 ± 0.01	58	0.98 ± 0.03^{ab}	58	3.31 ± 0.06^{bcd}	56	13.0 ± 0.29^{e}	
2012	60	1.13 ± 0.04	60	0.93 ± 0.06^{bc}	60	3.47 ± 0.12^{abc}	48	15.2 ± 0.51^{cd}	
2013	24	1.04 ± 0.04	24	0.79 ± 0.10^{cd}	23	3.31 ± 016^{bcd}	13	$17.8\pm1.53^{\rm ab}$	
2014	30	1.06 ± 0.05	30	0.73 ± 0.08^{d}	30	$2.57\pm0.12^{\rm f}$	23	12.7 ± 0.76^{e}	
2015	51	1.11 ± 0.04	51	0.88 ± 0.06^{bcd}	51	2.83 ± 0.11^{ef}	42	16.7 ± 0.82^{bc}	
2016	57	1.19 ± 0.05	57	1.00 ± 0.06^{ab}	57	3.54 ± 0.16^{abc}	49	16.8 ± 0.83^{bc}	
2017	48	1.08 ± 0.04	48	0.91 ± 0.07^{bc}	48	$\textbf{2.97} \pm \textbf{0.10}^{de}$	36	$13.3\pm0.60^{d\varepsilon}$	
2018	25	1.20 ± 0.08	25	1.12 ± 0.06^{a}	25	3.75 ± 0.20^{a}	24	19.4 ± 1.40^a	

Ns, P > 0.05; ***, P < 0.001; **, P < 0.01; *, P < 0.05.

LSB, litter size at birth; LSW, litter size at weaning; TLBW; total litter weight at birth; TLWW, total litter weight at weaning; D, Dorper; T, Tumele sheep.

values for LSB indicate that both genotypes are not that much prolific, but the value for LSW indicates their good mothering ability necessary to successfully raise lambs to weaning age.

Year of lambing was an important source of variation for all investigated reproductive traits except LSB. The influences of the year of lambing on reproductive traits are reported elsewhere (Mokhtari et al., 2010; Mohammadi et al., 2012; Boujenane et al., 2013). Reproductive traits are under the control of dam nutrition during and before pregnancy (Martin et al., 2004). Thus, the variation among season and among years may be explained by variation in the climate conditions and dependence of sheep to pastures, management and breeding conditions of mothers and lambs feeding in various years.

The TLBW and TLWW for male lambs were higher than female lambs. This result is consistent with several studies (Zhang et al., 2009; Kebede et al., 2012; Yavarifard et al., 2015). The superiority of males could be explained by the variability of the influence of sex hormones (androgen) on muscle development among males and females. Moreover, ewes which carry male lambs had higher cotyledon number and heavier placental weight than ewes carry female (Jawasreh et al., 2009). According to Oramari et al. (2011), the correlation between birth weight and weight of cotyledon is 0.64. This may be the other possible reason for the superiority of males than female counterparts.

TLBW is an important reproductive trait which measures the capacity of the dam to produce kid weight at birth regardless of their number (Mokhtari et al., 2010; Rashidi et al., 2011). Lambing season exerted a significant influence on TLBW and ewes lambing during the short rainy season had higher TLBW than ewes lambing in the other seasons. The mating season for ewes lambing during short rainy season was during dry season when there is no enough feed resource. This could be the possible reason for observed lower TLBW, as birth weight is under the influence of dam conditions.

4. Conclusion

In conclusion, most of the deaths occur during the pre-weaning period. Birth weight, birth type, sex and year of lambing were the most important risk factors for a survival rate of crossbred lambs. Tumele and Dorper x Tumele sheep are not that much prolific, but they had good mothering ability necessary to successfully raise lambs to weaning age. The current crossbreeding program which aims to improving growth performance had a positive influence on the survival of lambs. Improvement of environmental in the flock, special care for small lambs and indirect selection based on birth weight can lead to further survival improvement.

Declaration

Author contribution statement

Z. Tesema: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

B. Deribe, A. Kefale, M. Lakew, M. Tilahun and M. Shibesh: Performed the experiments.

N. Belayneh, A. Zegeye, G. Worku and L. Yizengaw: Performed the experiments; Contributed reagents, materials, analysis tools or data.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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