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Original article

Morphometrical evaluations of the integumental layers at different ages and regions of *Bakhtiari* sheep

B. Mobini

Department of Anatomical Sciences, College of Veterinary Medicine, Islamic Azad University, Shahrekord branch, P.O.Box:166. Shahrekord, Iran.

*Corresponding author; Department of Anatomical Sciences, College of Veterinary Medicine, Islamic Azad University, Shahrekord branch, P.O.Box:166. Shahrekord, Iran, Tel. +98 913-916-8248 - Fax: +98 381 3361060.

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ABSTRACT

For this study, 24 *Bakhtiari* sheep were selected and divided into four age groups: 1-10 days, 5-8 months, 1-2 years and 3 years and more. In each age group, six animals (3 each sex) were used. The specimens of eight different skin regions were collected. They were fixed in 10% formaldehyde solution and processed through routine paraffin embedding, cut at 5-7 µ and stained with H&E, Masson trichrome, Ayoub-Shklar, Verhoeff and Foot's method for reticulum. Morphometrical studies on these sections were carried out using light microscope and ocular micrometer respectively. Thickness of skin, epidermis and dermis, were measured in all the regions of skin in both sexes. The one-way ANOVA and Duncan's Multiple Range test were used to analyze the data and detect of significant differences. It was found that the thickness of total skin, epidermis, papillary layer and reticular layer of dermis varied among all the regions and was affected significantly by age and sex. The total skin thickness of different regions in neonate, young, young adult and old adult of *Bakhtiari* sheep ranged from 1197.81-1912.92, 1028.13-1861.90, 1712.08-2932.62, and 1585.24-2717.45 µ respectively.

1. Introduction

Skin is the interface between the organism and the environment and consists of an epidermis and a dermis (Dellmann, 1993; Mobini, 2012b). Epidermis which consists mainly of a multilayer of keratinocytes, is ectodermal origin and forms most of the appendages such as nail, sweat and sebaceous glands, hair and wool follicles (Junqueira and Camerio, 2003; Mobini, 2012b; 2012c; Mobini, 2013). Beneath of the epidermis is dermis, a thick and tough layer of connective tissue which extends to the hypodermis can be divided into a superficial papillary layer and a deep reticular layer (Dellmann, 1993; Mobini, 2012b). The sheep population in Iran is 50 million, comprising 26 genetic groups. Sheep are kept for meat, wool, milk and pelt (Osfoori and Fesus, 1996; FAO, 1998; Kiyanzad et al., 2003). In general, most of sheep breeds are multipurpose producing lambs, wool and milk. The breeds are named in relation to their place of origin or the tribe of the owner. Almost all sheep breeds of Iran are indigenous and have not yet been registered in formal breeding programs. Iran possesses 20 breeds of sheep. Approximately 65% of the total sheep population is considered pure and 35% crossbred. More than 96% of Iranian sheep are fat-tailed (Kiyanzad et al., 2003). *Bakhtiari* is an Iranian fat-tailed sheep which is raised in large numbers in Charmahal va *Bakhtiari* province in Iran where sheep production contributes significantly to the agricultural economy (Mobini, 2012a).

Quantity and quality characteristics of wool vary according to age, which is related to the growth physiology of sheep (Mobini, 2013). One of the main skin morphometric studies is evaluation of the integumental layers of various regions which have not been carried out in the most breeds especially in *Bakhtiari* sheep. Therefore, the purpose of this study was to evaluate the morphometrical changes in different areas of the skin and its relationship with age in *Bakhtiari* sheep in both sexes.

2. Materials and methods

Twenty-four clinically healthy *Bakhtiari* sheep were selected according to their phenotypic features and divided into four age groups: 1-10 days (neonate), 5-8 months (young), 1-2 years (young adult) and 3 years and more (old adult). In each age group, six animals (3 each sex) were used. The specimens of eight different skin regions were collected. Samples of skins each of 3 cm^2 were taken from the following eight regions on each sheep: 1- belly, 2- neck, 3- leg, 4- rump, 5- flank, 6- forearm, 7- shoulder and 8- hip. They were fixed in 10 percent neutral buffered formaldehyde. Tissues were stained with haematoxylin and eosin, Masson's trichrome, Ayoub-Shklar, Foot's method for reticulum (Kiernan, 1999), and Verhoeff's (Mallory, 1968). By using ocular micrometer, thickness of skin, epidermis and dermis, were measured in all the four age groups and in all the regions of skin. Data were finally analyzed by one-way ANOVA, using the SPSS statistic software version 18. Duncan's Multiple Range test was also used to detect significant differences ($P<0.05$).

3. Results

The thickness of the skin varied among the regions and different age groups studied. The maximum thickness of skin (1912.92 and 2717.45 µ) was recorded in neonates and old adult sheep respectively on the neck followed by 1861.90 µ in young on the leg and 2932.62 µ in young adult sheep on flank region. The minimum thickness of skin (1197.81 and 1712.08 µ) was recorded in neonates and young adult sheep respectively on the forearm followed by 1028.13 µ in young on the shoulder and 1585.24 µ in old adult sheep on leg region.

The thickness of epidermis varied among the regions and different ages. The maximum thickness of epidermis (50.71 and 65.71 µ) was recorded in young and young adult sheep respectively on the rump followed by 33.91 µ in neonatal sheep on the leg and 80.36 µ in old adult sheep on forearm region. The minimum thickness of epidermis (20.77 µ) was recorded in neonates on the flank followed by 23.96 µ in young on the shoulder and 30.42 µ in young adult sheep on neck and 41.17 µ in old adult sheep on rump region.

4. Discussion

The thickness of papillary layer of dermis varied among the regions and different age groups. The maximum thickness of papillary layer of dermis (1104.17 and 1779.17 μ) was recorded in neonatal and old adult sheep respectively on the neck followed by 900 μ in young sheep on the leg and 1491.67 μ in young adult sheep on shoulder region. The minimum thickness of papillary layer of dermis (778.57 and 716.67 μ) was recorded in neonatal and old adult sheep respectively on the leg followed by 416.67 μ in young sheep on the belly and 920.83 μ in young adult sheep on forearm region.

The thickness of reticular layer of dermis also varied among the regions and different age groups studied. The maximum thickness of reticular layer of dermis (775.00 and 1145.83 μ) was recorded in neonatal and young sheep respectively on the neck followed by 1495.83 μ in young adult sheep on the flank and 1025.00 μ in old adult sheep on shoulder region. The minimum thickness of reticular layer of dermis (312.50 and 660.71 μ) was recorded in neonatal and young adult sheep respectively on the belly followed by 341.67 μ in young sheep on the forearm and 812.50 μ in old adult sheep on leg region.

Table 1
Thickness of skin layers (μ) at different regions of 1-10 days ages of *Bakhtiari* sheep.

Region		Dermis		
		Epidermis	**Papillary layer**	**Reticular layer**
Belly	M	26.07±11.89	766.67±38.19[a]	233.33±62.91
	F	28.21±6.07	966.67±137.69[a]	391.67±212.62
	M±F	27.14±9.14	866.67±142.01	312.50±164.89
Neck	M	35.71±17.18	950.00±139.19	808.33±187.64
	F	31.00±3.79	1258.33±80.36	741.67±101.04
	M±F	33.75±13.12	1104.17±197.11	775.00±139.64
Leg	M	36.67±18.03	837.50±228.67	418.75±158.61
	F	30.36±3.93	700.00±90.14	583.33±262.60
	M±F	33.91±13.78	778.57±185.08	489.29±208.09
Rump	M	30.83±16.49	883.33±200.52	300.00±108.97[a]
	F	25.50±5.97	1300.00±390.51	608.33±76.38[a]
	M±F	28.93±13.61	1091.67±359.40	454.17±188.69
Flank	M	22.50±6.12	1125.00±175.00	641.67±150.69
	F	18.00±6.94	900.00±217.94	525.00±152.07
	M±F	20.77±6.57	1012.50±215.49	583.33±149.72
Forearm	M	46.67±17.86[a]	741.67±80.36	400.00±241.09
	F	12.50±3.95[a]	866.67±80.36	325.00±66.14
	M±F	31.14±22.00	804.17±99.27	362.50±163.36
Shoulder	M	33.33±12.42	800.00±278.39	700.00±501.87
	F	24.00±7.20	783.33±123.32	608.33±224.07
	M±F	29.09±11.03	791.67±192.79	654.17±351.22
Hip	M	40.83±12.42[b]	741.67±94.65	608.33±374.44
	F	14.00±4.87[b]	858.33±52.04	291.67±38.19
	M±F	28.64±16.82	800.00±93.54	450.00±294.53

F – Females; M – Males; Same small letters within a column differ significantly (P<0.05). All values are in mean ± S.D.

In neonatal sheep, the thickness of epidermis in forearm and hip skin showed significant differences between males and females, so that the maximum and minimum thickness of epidermis (46.67 and 12.50 μ) was found in forearm of males and forearm of female sheep respectively.

The only significant difference in thickness of papillary layer of dermis, between males and females was observed in the belly skin that in females were thicker than males in the sheep aged 1-10 days. In neonatal sheep, the only significant sex-related difference in reticular layer thickness was observed in the rump skin that in females were thicker than males (Table 1).

In young sheep, the thickness of epidermis in the belly, leg, forearm and hip skin were affected by sex, and in males were thicker than females. In the 5-8 months age group, sex related differences in the thickness of papillary layer of dermis were observed for the rump and hip skin. This layer in males was thicker than females. The only significant sex- based difference for the reticular layer thickness in the sheep aged 5-8 months was observed in the rump skin that in females were thicker than males (Table 2).

Table 2
Thickness of skin layers (μ) at different regions of 5-8 months ages of *Bakhtiari* sheep.

Region		Dermis		
		Epidermis	**Papillary layer**	**Reticular layer**
Belly	M	53.75±15.2[a]	491.67±301.39	1108.33±38.19
	F	35.00±11.73[a]	341.67±62.91	700.00±338.19
	M±F	45.71±16.45	416.67±211.34	904.17±310.41
Neck	M	26.25±11.59	591.67±208.17	991.67±251.66
	F	27.50±6.92	666.67±224.07	1300.00±238.48
	M±F	26.92±8.96	629.17±197.75	1145.83±276.77
Leg	M	50.00±13.95[b]	825.00±246.22	1016.67±128.29
	F	29.17±11.80[b]	975.00±163.94	825.00±288.31
	M±F	41.07±16.52	900.00±204.33	920.83±225.51
Rump	M	37.08±9.80	925.00±66.14[a]	508.33±94.65[a]
	F	60.94±26.39	550.00±43.30[a]	841.67±128.29[a]
	M±F	50.71±23.70	737.50±211.39	675.00±208.57
Flank	M	26.67±3.03	683.33±170.17	441.67±38.19
	F	22.50±4.18	666.67±80.36	625.00±139.19
	M±F	24.58±4.11	675.00±119.37	533.33±135.71
Forearm	M	48.75±13.30[c]	883.33±184.28	333.33±28.87
	F	27.14±9.51[c]	633.33±57.73	350.00±43.30
	M±F	37.12±15.64	758.33±183.48	341.67±34.16
Shoulder	M	27.50±19.75	600.00±90.14	408.33±128.29
	F	20.42±5.34	608.33±142.16	391.67±76.38
	M±F	23.96±14.28	604.17±106.56	400.00±94.87
Hip	M	41.25±13.67[d]	1050.00±222.20[b]	725.00±50.00
	F	22.50±3.06[d]	325.00±129.90[b]	583.33±212.62
	M±F	32.73±13.89	687.50±429.17	654.17±158.44

F – Females; M – Males; Same small letters within a column differ significantly (P<0.05). All values are in mean ± S.D

In the 1-2 years sheep, with the exception of the leg and shoulder, the thickness of epidermis in the other skin regions were more in females than males. In the sheep aged 1-2 years, the males had significant thicker of papillary layer of dermis in all the skin regions than females. In young adult sheep, the thickness of reticular layer of dermis in the belly, neck and forearm skin were also affected by sex, and in males were thicker than females (Table 3).

In the old adult sheep, males had thicker epidermis in forearm and hip skin than females, whereas the thickness of epidermis in the leg skin was more in females than males. In the 3 and more years sheep, females had thicker papillary layer of dermis in neck, flank, shoulder and hip skin than females whereas, it was more in males than females for the forearm skin. The only significant sex- based difference for the reticular layer thickness in the sheep aged 3 and more years was observed in the flank skin that in males were thicker than females (Table 4).

The thickness of the skin was varied among all the regions and different age groups studied. This finding is in agreement with the results of previous studies (Genkovski and Gerchev, 2007; Mir Shabir et al., 2011). In neonatal, young, young adult and old adult sheep, the total skin thickness ranged from 1197.81 to 1912.92 μ, 1028.13 to 1861.90 μ, 1712.08 to 2932.62 μ, and 1585.24 to 2717.45 μ, respectively. Genkovski and Gerchev (2007) reported

that in Tsigai ewes, the total skin thickness ranged from 2703.5 to 2994.7 µ (12). Abbasi et al. (2008) observed that the total skin thickness was 2400 µ in Lori sheep. The total skin thickness in native and hybrid of Merino sheep was 2897.8 µ to 3507.2 µ (Ozfiliz et al., 2002).

The total skin thickness in Kuibyshev and Romney Marsh rams aged over 3 years were 4248 and 4764 µ, respectively (Builov et al., 1976). The total thickness of flank skin of some indigenous types of Bulgarian sheep aged 2 years was averaged 2269, 2308, 1971, 2007 and 1748 µ, respectively (raichev and Khristova, 1990).

The thickness of skin had increased as age advanced as reported by Mir Shabir et al. (2011) in madras red sheep, Fayez et al. (1976) in Awassi fat-tailed sheep, and Saxena et al. (1994) in cattle. Thickness of skin increased rapidly from young age to adult than from neonatal age to young and from young adult to old adult age, whereas thickness of skin in madras sheep increased rapidly from neonatal age to young than from young to adult age (Mir Shabir et al., 2011). The thickness of epidermis was varied in different regions and in all the four age groups. This finding is in agreement with the results of previous studies (Abbasi et al., 2008; aktas and Daglioglu, 2009; Britt et al., 1985; Butler, 1991; Genkovski and Gerchev, 2007; kurtdede and Asti, 1999; Mir Shabir et al., 2011). When the percentages of epidermis in old adult Bakhtiari sheep (1.72 to 3.54 %) were compared with those in adult Tsigai ewes (0.65 to 0.71 %), it was determined that the number of cell layers constituted this layer in Bakhtiari sheep was more than Tsigai ewes (Genkovski and Gerchev, 2007).

Table 3
Thickness of skin layers (µ) at different regions of 1-2 years ages of *Bakhtiari* sheep.

Region		Dermis		
		Epidermis	**Papillary layer**	**Reticular layer**
Belly	M	22.50±4.68 [a]	1200.00±360.55	1083.33±137.69[a]
	F	63.21±21.20[a]	1100.00±170.78	343.75±106.80[a]
	M±F	46.25±26.32	1142.86±246.52	660.71±410.25
Neck	M	22.92±5.10[b]	1225.00±173.20	1266.67±52.04[b]
	F	37.92±9.28[b]	1233.33±101.04	858.33±123.32[b]
	M±F	30.42±10.60	1229.17±126.90	1062.50±239.14
Leg	M	56.50±6.75[c]	1500.00±114.56[a]	833.33±274.24
	F	33.21±8.38[c]	1125.00±84.16 [a]	706.25±134.44
	M±F	42.92±14.09	1285.71±219.31	760.71±196.77
Rump	M	47.08±10.89[d]	1866.67±339.42[b]	1033.33±284.31
	F	79.69±31.12[d]	1000.00±216.51[b]	800.00±125.00
	M±F	65.71±29.11	1433.33±538.67	916.67±234.34
Flank	M	35.00±7.42[e]	1491.67±212.62	1766.67±341.26
	F	60.00±15.47[e]	1283.33±80.36	1225.00±90.14
	M±F	49.29±17.74	1387.50±183.54	1495.83±371.29
Forearm	M	35.42±12.19	1250.00±156.12[c]	1066.67±194.19[c]
	F	38.75±17.08	591.67±118.14[c]	441.67±14.43[c]
	M±F	37.08±14.26	920.83±381.25	754.17±363.80
Shoulder	M	63.33±16.56[f]	2050.00±478.93[d]	1000.00±229.13
	F	35.42±15.44[f]	933.33±262.60[d]	816.67±200.52
	M±F	49.38±21.11	1491.67±702.44	908.33±217.18
Hip	M	18.75±8.18 [g]	1283.33±62.91[e]	958.33±314.58
	F	41.79±17.30[g]	766.67±276.51[e]	725.00±175.00
	M±F	31.15±17.90	1025.00±335.04	841.67±261.09

F – Females; M – Males; Same small letters within a column differ significantly (P<0.05). All values are in mean ± S.D.

The thickness of dermis was varied in different regions and in all the four age groups. From neonatal to adult age group sheep, thickness of both papillary and reticular layers of the dermis was found to be increased in all the regions in the present study, which correlates to the findings of Mir Shabir et al. (2011) in madras red sheep.

In the present study, the maximum thickness of dermis was found in 1-10 days and 3 years and more ages in the neck (1879.17 and 2666.67 µ respectively), in young age in the leg (1820.83 µ) and in young adult sheep in the flank region (2883.33 µ). The maximum thickness of dermis in neonatal, young and adult madras sheep was 1347.52, 2456.00 and 2902.00 µ, respectively in the neck and the minimum thickness was 900.33, 1427.00 and 1486.16 µ, respectively on thorax ventral region of skin (Mir Shabir et al., 2011). The average initial thickness of dermis in sheep breeds fetuses was 143.55 and 1770.00 µ (aktas and Daglioglu, 2009).

The thickness of papillary layer of dermis was from 920.83 -1491.67 µ for young adult sheep to 716.67-1779.17 µ for old adult Bakhtiari sheep. raichev and Khristova (1990) observed that in Duben, Kotel, Stranja, Sakar and Central Rodopi ewes, the thickness of papillary layer of flank skin averaged 1579, 1670, 1500, 1494 and 1229 µ respectively. The percentages of papillary layer were found to be higher in Tsigai ewes (67.24-68.77 %) when compared to the old adult sheep (45.21 to 65.47 %) in the present study. on the other hand, the percentages of reticular layer in old adult Bakhtiari sheep (32.66-51.25 %) were more than Tsigai ewes (30.49-32.08 %) that indicated the dermis of Bakhtiari sheep skin consisted of more reticular layer and less papillary layer (Genkovski and Gerchev, 2007).

Table 4
Thickness of skin layers (µ) at different regions of 3 years and more ages of *Bakhtiari* sheep.

Region		Dermis		
		Epidermis	**Papillary layer**	**Reticular layer**
Belly	M	76.88±83.83	916.67±118.14	891.67±52.04
	F	49.06±18.89	1066.67±462.56	741.67±160.73
	M±F	62.97±60.44	991.67±312.92	816.67±134.78
Neck	M	59.06±22.64	1458.33±280.99[a]	875.00±486.70
	F	42.50±7.44	2100.00±5.00[a]	900.00±5.00
	M±F	50.78±18.39	1779.17±393.84	887.50±308.14
Leg	M	45.71±15.86[a]	575.00±43.30	741.67±146.49
	F	66.43±18.48[a]	858.33±251.66	883.33±224.07
	M±F	56.07±19.73	716.67±223.98	812.50±186.25
Rump	M	35.00±12.89	1891.67±137.69	791.67±267.32
	F	48.21±19.46	1091.67±600.17	925.00±694.62
	M±F	41.17±17.08	1491.67±586.23	858.33±476.36
Flank	M	40.83±4.92	575.00±5.00[b]	1248.33±7.64[a]
	F	69.17±32.74	1358.33±350.30[b]	633.33±87.80[a]
	M±F	55.00±26.78	966.67±482.88	940.83±341.43
Forearm	M	96.43±35.53[b]	1983.33±242.81[c]	875.00±363.15
	F	64.29±10.58[b]	1083.33±226.84[c]	1066.67±142.16
	M±F	80.36±30.20	1533.33±535.88	970.83±268.06
Shoulder	M	63.93±15.06	725.00±5.00 [d]	950.00±5.00
	F	51.07±26.17	1475.00±139.19[d]	1100.00±278.39
	M±F	57.50±21.57	1100.00±420.13	1025.00±194.32
Hip	M	57.50±16.27[c]	1083.33±194.19[e]	1058.33±128.29
	F	34.69±22.14[c]	2191.67±123.32[e]	766.67±202.07
	M±F	45.33±22.30	1637.50±624.25	912.50±220.08

F – Females; M – Males; Same small letters within a column differ significantly (P<0.05). All values are in mean ± S.D.

5. Conclusion

In conclusion, sex showed significant effect on the thickness of the skin layers in all the age groups. The total skin thickness and its layers varied among the regions and different age groups.

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