

The stressing effect of shearing in mid-pregnancy of Zandi ewes on live weight and body sizes of twin lambs at the time of birth

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ABSTRACT

This research investigated the effect of stress of mid-pregnancy shorn Zandi ewes on the live birth weight and body sizes of twin lambs. A total of 96 Zandi ewes, confirmed pregnant with twins, were randomly allocated on day 80 of pregnancy into 4 groups, each group containing 6 replicates (each replicate containing 4 sheep). The experimental groups included: 1. Control, 2. Crutched, 3. Sham-Shorn, and 4. Shorn. Blood cortisol concentrations of the ewes in the experimental shorn treatment group increased significantly compared to control ($P < 0.05$). The average length of pregnancy in the ewes of the shorn treatment group was 2 and 1.5 days longer than the control and Sham-Shorn groups ($P < 0.05$). Lambs born from ewes given the shorn treatment were heavier at birth as compared to the lambs of the other treatments, as well as having longer body lengths, arms, and legs ($P < 0.05$). In general, the results showed that the ewes that were shorn in mid-pregnancy had a higher cortisol concentration, and it had a significant effect on the birth weight and sizes of the lambs compared to other treatments.

Keywords: Lamb, Live weight, Shearing, Stress, Zandi ewes

INTRODUCTION

The survival of lambs is affected by lamb birth weight and size as well as by the maternal behavior of the ewe. The main reasons for death of lambs include difficult birth, starvation, lack of acceptance by the mother ewe, and low birth weight (Hinch and Brien, 2013; Slozhenkina *et al.*, 2020). Lamb birth weight has a linear relationship with survival rate, with the light lambs have the highest mortality rate (Nowak and Piondron, 2006). It was reported that the birth weight range suitable for lamb survival was from

3 to 5.5 kg for New Zealand hill sheep (Dalton *et al.*, 1980). In previous studies, it has been determined that lambs with a birth weight of less than 3 kg are at a high risk of starvation, and exposure to starvation due to insufficient body reserves and reduced body strength. Additionally, lambs that weigh more than 5.5 kg experience more starvation, birth defects, cardiac complications and difficult birthing (Nowak and Piondron, 2006). Kerslake *et al.* (2005) noted that the average weight of twin and triplet lambs that died within the first 3 days after birth was lower than that of lambs that survived this period.

The results of previous studies have shown that stressors during pregnancy of ewes, such as poor body condition, uncomfortable temperatures, or even common management methods such as transportation or shearing, suppress the animal's natural behavior and optimal fetal growth and can change the physiological responses of lambs after birth (Rooke *et al.*, 2017).

The first sign of prenatal stress is the changes in the fetal brain, which indicate functional changes in synaptic transmission to cope with the problems in fetal life (Petit *et al.*, 2015). Stress during the prenatal period can alter fetal growth and development through the transfer of glucocorticoids from the maternal circulation to placental tissues (McMillen and Robinson, 2005). Transfer of glucocorticoids from the placenta is regulated by 11 β -hydroxysteroid dehydrogenase type 2 (11 β -HSD-2), which converts cortisol to its physiologically inactive form, i.e. cortisone (Seckl, 1998).

11 β -HSD-2 activity creates an incomplete barrier that allows 10–20% of active maternal glucocorticoids to pass (Seckl and Meaney, 2006). In addition, maternal malnutrition and increased glucocorticoids can decrease placental activity due to 11 β -HSD-2. Therefore, a change in the maternal cortisol concentration causes a change in the fetal plasma cortisol concentration (Seckl and Meaney, 2006; Otten *et al.*, 2004).

The concentration of cortisol in the fetus increases gradually beginning 10 to 15 days before birth, and this is accelerated 3 to 5 days before birth by a large and rapid increase (Silver and Fowden, 1988). Fowden (1998) has shown that the increase in cortisol concentration hinders fetal growth through the separation and maturation of fetal tissues.

There are conflicting reports about the effect of stress on pregnant ewe.. Some researchers report that such stress leads to decrease weight and size of lambs, while other researchers report the opposite, that these stresses can cause positive effects such as increase birth weight and increase weight gains.

Shearing affects the shorn animal in several ways. This includes stress from starvation before shearing, exposure of sheep to human activities, the process of separating and collecting sheep, the stress caused by the loud noise of the electric shearing machine, the cold caused by

detachment of the body's woolly coat and an increase in plasma cortisol concentration (Hargreaves and Hutson, 1990).

On the other hand, the results of some research show that shearing in pregnant ewes causes a change in the behavior of the ewes leading to an increased birth weight of the lambs and improved subsequent performance of the lamb, such as higher weaning weight (De Barbieri *et al.*, 2018).

Guyoti *et al.* (2015) stated that shearing of ewe on the 74th day of pregnancy is effective for better growth of lambs after birth and reduce of prenatal mortality. Revell *et al.* (2002) have reported that although the birth weight of lambs with wool picking increased on the 70th day of pregnancy (to the amount of 0.5 kg), the weight of the placentas on the 140th day of pregnancy was similar in shorn and control groups. They concluded that the increase in the birth weight of lambs due to shearing in mid-pregnancy can be due to the strengthening of the passage of glucose through the placenta.

Previous studies have shown that the exposure of ewes to stressful factors in mid-pregnancy causes changes in hormones and the weight of twin lambs, with the ultimate effect on lambs being decrease or increase lamb birth weight and size. The present study addresses this controversy and aims to determine the effect of stressors in the mid-pregnancy of Zandi ewes on live weight and body sizes of twin lambs at birth.

MATERIALS AND METHODS

Ewes

This research was conducted in a semi-nomadic sheep and goat breeding farm, located in Shahryar city in the Southwest of Tehran province in Iran, from the middle of September 2018 to the middle of March 2019.

A total of 290 Zandi ewes of different ages were synchronized for 13 days of the estrus period using an intravaginal device containing progesterone called CIDR. On the day when the CIDRs were removed from the vagina of the ewes, 400 units of PMSG hormone were injected deep into each ewe to induce estrus, ovulation, and simultaneous estrus, and at the same time, the rams of the Zandi breed were introduced to

the herd with a ratio of 1 ram to 5 ewes. Crossings were recorded individually and were recorded daily, and with the help of the coloring mark, each ram stayed in the herd for 5 days (from October 1 to 5, 2018). After 5 days, the Zandi rams were removed from the herd and a few other Zandi rams with a ratio of 1 ram to 25 ewes were entered into the herd and replaced. Ewes that were colored at this stage and mated with rams were removed from the flock. On the 59th day of the rearing period (P59), it was determined that 96 ewes were pregnant with twins and these were selected to continue the experiment.

Ewes carrying twin lambs were randomly divided into 4 treatments, each treatment containing 6 replicates (each replicate containing 4 sheep). The experimental treatments included 1. Control, 2. Crutched, 3. Sham-Shorn, and 4. Shorn. All ewes were treated on the 80th day of pregnancy (December 20, 2018).

The minimum and maximum values of air temperature after shearing in December 2018 were 1.7 and 11.8 °C, respectively. The minimum temperature decreased below 0°C for two days (on the second and sixth days after shearing). The average rainfall on the seventh day after shearing was 0.1 mm.

The ewes of the group of crutched, sham-shorn, and shorn were removed from the pasture that was the pasture of crops 24 hours before the treatment. The ewes in the crutched treatment were managed in the same way as the shorn treatment, but the duration of the procedure was shorter. The sham-shorn treatment included all the conditions and methods related to complete shearing (approximately 5 minutes) and the shearing machine was turned on to produce the necessary sound, but the person picking the wool by hand was careful not to separate any wool fibers from the body. The cover, which left 5-7 mm long fibers on the body, was used for shorn and crutched treatments (Dabiri *et al.*, 1995). All the ewes had approximately 6 months of fiber growth. The wool of the shorn ewes was weighed, and the live weight of the ewes was corrected based on the weight of the wool. The wool obtained from the crutched group was not weighed. During the rest of the pregnancy period, all ewes were kept as a single management group under semi-industrial rearing conditions.

On the 80th day of pregnancy, 3 ewes were randomly selected from each replicate and blood samples were taken immediately before treatment ($t=0$) at 10, 20, 30, 60, and 90 minutes after treatment. Blood samples were taken using venoject tubes containing sodium heparin in the amount of 10 ml. The blood samples were immediately transferred to the laboratory with ice and centrifuged at 3000 rpm for 10 minutes. Blood plasma after separation was kept at -20°C. Plasma was analyzed by radioimmunoassay to determine total cortisol concentration. GammaCoat™ antiserum analysis showed a 100% reactivation reaction with cortisol (Anonymous, 2003). The coefficient of variation between the announced tests was 7.0% and the coefficient of variation within the test was 9.2%.

The live weight of ewes was measured on days 79, 112, and 140 of pregnancy and day 42 of lactation. On the 130th day of pregnancy, all the ewes were crutched to prepare for delivery. The ewes were randomly divided into lambing pens on the 140th day of pregnancy. During the lambing period, the ewes were checked 3 times a day.

Lambs

During the first 12 hours of birth, the lambs were numbered and weighed and their body sizes were measured. The body sizes of the lambs were body length (CRL), chest (immediately after the hands), front leg length (shoulder to the tip of the hoof on the left leg), and hind leg length (thigh joint to the tip of the hump on the left leg). The color of meconium was observed based on 4 degrees from 0 to 3, (0=white, 1=light yellow, 2=yellow, 3=dark yellow/orange) by the method of collecting and placing in white plastic containers, as an indicator of stress level generated during lambing was measured (Oliver *et al.*, 2001). The live weight of the lambs was measured again on the 42nd and 94th days after birth.

Statistical Analysis

All the continuous variance data were analyzed using the model GLM in SAS software. The data that did not have a normal distribution were analyzed by the Kruskal-Wallis test using the non-parametric method (NPAR1WAY) in SAS software. Classified data (meconium color grade) was analyzed using GENMOD method

and SAS software. Data recorded over time were analyzed in the software with the mixed model and repeated data analysis method. Lamb survival data was considered a binomial trait and was transformed by the logit method and analyzed using GENMOD method in SAS software.

Cortisol data were not normally distributed (Anderson-Darling normality test, $P < 0.05$), and $X^{0.5}$ data transformation was used to normalize the data distribution, shapes represent means and standard error of raw data as well as P values.

Lamb live weight on day 42 after birth (L42), body and arm length of lambs did not have a normal distribution (Anderson-Darling normality test, $P < 0.05$). Distribution of body length and live weight of lambs on day 42 after birth (L42) were normalized using x^2 data transformation, and arm length was normalized using \log_e data transformation method. The length of the pregnancy period of the ewes did not have a normal distribution and was not normalized by any of the data transformation methods. Therefore, the median of pregnancy length data was presented descriptively.

The analysis of body sizes of lambs (CRL, chest circumference, arm length, and leg length) was processed in two ways, with and without birth weight as a covariate. The purpose of this study was to investigate whether the differences in body sizes were proportional to birth weight or whether these data indicated the differences in the body shape of the lambs. In addition, the live weights of lambs at birth, age 42 days (L42), and age 94 days (L94) were corrected for the body weight of their mothers on days 140 of pregnancy (P140), 42 and 94 of lactation (L94,42).

RESULTS

The responses of plasma cortisol in ewes in the shorn group and the crutched group were similar (Figure 1). Control group ewes had lower plasma cortisol concentration ($P < 0.05$) compared to other treatments within 10 minutes after treatment ($P < 0.05$; Figure 1 and Table 1). The ewes of the sham-shorn group had lower plasma cortisol concentration compared to the ewes of the shorn group 20 and 30 minutes after applying the treatment ($P < 0.05$). Ewes in the sham-shorn group had lower plasma cortisol concentration 90 minutes after the treatment compared to the

control group and shorn group ewes ($P < 0.05$).

At 10 minutes after applying the treatment, the ewes of the control group had lower cortisol as compared to the rest of the treatments ($P < 0.05$). Compared to the other groups, the ewes of the control group spent more time reaching the maximum amount of cortisol in the body ($P < 0.05$; Table 1). The ewes of the sham-shorn group had lower cumulative cortisol responses compared to the crutched ewes and the shorn ewes ($P < 0.05$).

The results related to the effect of experimental treatments on the live weight of ewes during pregnancy and lactation are presented in Table 2. According to the results of experimental treatments, it did not affect the live weight of ewes during pregnancy and lactation.

The results related to the effect of the experimental treatments on the average duration of pregnancy in ewes are presented in Table 3. The average duration of pregnancy in shorn group ewes was 2 and 1.5 days longer than control and sham-shorn group ewes ($P < 0.05$). The durations of the pregnancy period in the control, crutched and sham-shorn groups were almost similar.

The results related to the effect of the experimental treatments on the live weight of the lambs at the time of birth and during lactation (days 42 and 94) as well as the survival percentage of the lambs are presented in Table 4. The birth weight of lambs in the shorn group were heavier than those in the control group, the crutched group, and the sham-shorn group ($P < 0.05$). The treatment had no effect on the live weight of lambs on days 42 or 94 of lactation. The ratio of lambs with a birth weight lower than, equal to, or greater than the optimal birth weight range for survival (3.5-5.5 kg) was the same among different treatment groups.

The effect of experimental treatments on the body sizes and level of meconium of the new born lambs are presented in Table 5. The lambs born from the shorn group had longer body lengths, arms and legs as compared to lambs born ifrom the control and other treatments ($P < 0.05$). No significant difference was observed between the different treatments for the breast circumference and the color of the meconium of the lambs.

DISCUSSION

In the present experiment, crutching and shearing caused similar increased in plasma cortisol, and it took longer in the shorn group to reach the baseline value. The most common response by the autonomic nervous system to a stressor is the activation of the hypothalamic-pituitary axis (HPA) (Matteri *et al.*, 2000). Nerve cells in the paraventricular zone release the hormones corticotrophin (CRH) and arginine-vasopressin (ANP), which cause the anterior part of the pituitary gland to produce adrenocorticotrophic hormone (ACTH) (Tilbrook *et al.*, 2000). Under the control of ACTH, glucocorticoids are produced by the adrenal cortex (Ferguson and Hoenig, 2001).

Astrup *et al.* (1988) reported in their research results that 8 days after shearing of ewes, the amount of corticosteroid was lower compared to 3 days after shearing. They stated that the concentration of corticosteroids decreased over time in response to an acute stressor, but if the stressor was chronic, the concentration of corticosteroids did not change over time or increase.

Mendoza *et al.* (2000) stated that acute re-

sponse to stress is observed when plasma cortisol concentration reaches its pre-treatment levels. Since in the present experiment, after 90 minutes of treatment, the concentration of cortisol decreased in the experimental treatments, an acute response to stress may be caused by the stressors used in this experiment.

In the current research, the live weight of ewes during pregnancy was not affected by any of the treatments. This is consistent with a prior study that the live weight of ewes during the lactation period is usually not affected by shearing during pregnancy (Kenyon *et al.*, 2002). However, Morris *et al.* (2000) indicated that the shorn ewes on days 5, 100, or 130 of pregnancy had lower live weight compared to non-shorn ewes on day 45 of lactation. In contrast to those researches, Morris *et al.* (2000) and Smeaton *et al.* (2000) reported that shearing increases the live weight of ewes during the pregnancy period.

In the present experiment, since the concentration of plasma cortisol increased because of stress and then reached the levels before the application of the treatment, the stressors created by the weight loss of the ewes possibly do not have a significant effect.

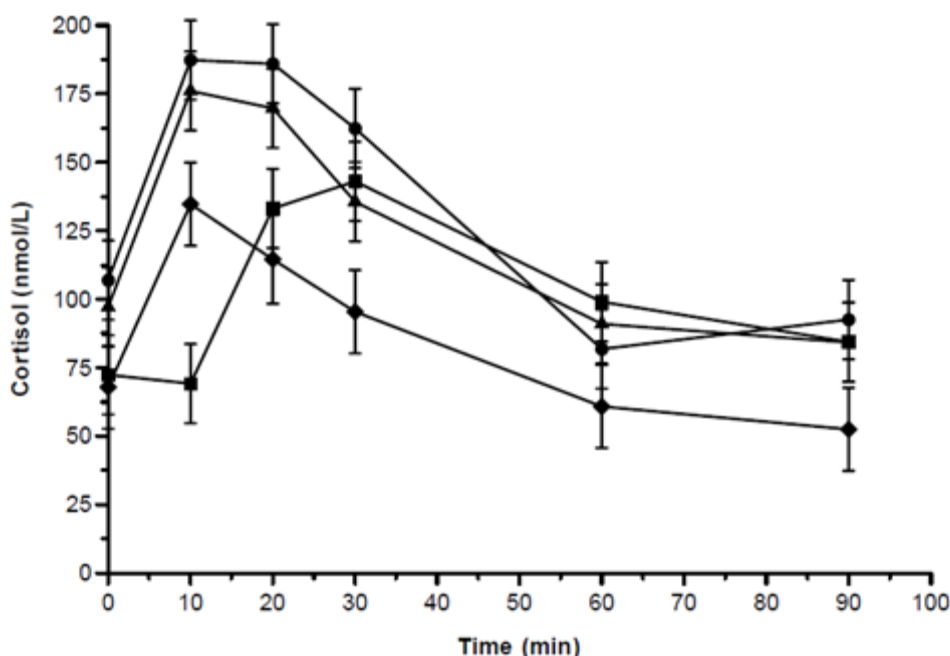


Figure 1. The effect of experimental treatments on plasma cortisol concentration of twin ewes before 0 and immediately 10, 20, 30, 60, and 90 minutes after treatment (80th day of pregnancy)

■= control, ▲= crutched, ◆= sham-shorn, and ●= shorn

Table 1. The effect of experimental treatments on plasma cortisol concentration 10 minutes after treatment (nmol/L), the time to reach the maximum level (minutes) and the concentrated cortisol response (nmol/L) (least square mean \pm standard error)

Treatment	n	Cortisol concentration after 10 minutes ¹ (nmol/L)	The time required to reach the maximum concentration (minutes)	The area under the response curve ¹ (nmol/L)
Control	18	4.21 ^c \pm 0.2 (69.2)	30.2 ^a \pm 2.7	4.62 ^{ab} \pm 0.1 (105.4)
Crutched	18	5.08 ^a \pm 0.2 (176.0)	14.5 ^b \pm 2.7	4.69 ^a \pm 0.1 (118.3)
Sham-shorn	18	4.84 ^b \pm 0.2 (134.7)	12.8 ^b \pm 2.7	4.34 ^b \pm 0.1 (79.8)
Shorn	18	5.19 ^a \pm 0.2 (187.4)	16.4 ^b \pm 2.7	4.78 ^a \pm 0.1 (126.2)

Different averages in each column indicate a significant difference at the 5% level ($P < 0.05$).

¹ The data were transformed using the $X^{0.5}$ method and the transformed means are shown in parentheses.

Table 2. The effect of experimental treatments on live weight (kg) of twin ewes (means \pm least squares and standard deviation)

Treatment	n	Live weight of ewe (kg) ¹				
		P79	P112	P140	L42	L94
Control	24	56.3 \pm 1.6	59.8 \pm 1.7	63.6 \pm 1.9	51.5 \pm 1.3	51.3 \pm 1.3
Crutched	24	55.3 \pm 1.5	60.1 \pm 1.5	65.0 \pm 1.7	50.3 \pm 1.1	51.5 \pm 1.2
Sham-shorn	24	56.3 \pm 1.6	58.3 \pm 1.7	63.3 \pm 1.9	52.6 \pm 1.3	52.0 \pm 1.3
Shorn	24	55.4 \pm 1.4	58.9 \pm 1.5	62.8 \pm 1.7	50.7 \pm 1.1	50.9 \pm 1.2

P79: 79th day of pregnancy; P112: 112th day of pregnancy; P140: 140th day of pregnancy; L42: 42nd day of lactation and L94: 94th day of lactation.

¹ The live weight of ewe from the 112th and 140th day of pregnancy and the 42nd and 94th day of lactation were adjusted based on the weight on the 80th day of pregnancy.

According to the present results, the average length of pregnancy in shorn group ewes was 2 and 1.5 days longer than control and sham-shorn group ewes, respectively. Similar to our results, Cam and Kuran (2004) concluded that shearing of ewes during pregnancy can increase the pregnancy period (about 0.5 to 1.5 days). They concluded that during shearing, a part of the metabolism in the ewes is used to deal with the stress and to warm the body, which causes a slight slowdown in the development of the fetus in the pregnant ewes, and as a result, the duration of the pregnancy period will increase slightly.

De Barbieri *et al.* (2018) stated that shearing of pregnant ewes has different effects on the lamb's birth weight. In their experiments, they found that the lambs born from ewes that were shorn in mid-pregnancy compared to lambs born from ewes that were shorn after parturition had higher birth weight and survival rate (De Barbieri *et al.*, 2018), which is consistent with the results of the present experiments.

The shorn ewes show a different feeding behavior such that they spend more time eating during the day (compared to the night), stand more time, and spend less time on the ground during the night than the non-shorn ewes (Hutchinson and McRae, 1969; Fez *et al.*, 2021). The ewes that are sheared in the mid- and late-pregnancy are more interested in staying in the stall, and at the time of delivery, a higher percentage of these ewes give birth in the stall, and as a result, the chance of their lambs' survival increases (Gregory, 1995).

In the research of Clarke *et al.* (1997), it has been determined that the mother's adaptation because of being shorn during pregnancy leads to the increase of lamb's survival and supply of glucose to the fetus. They stated that plasma glucose concentration increased in late pregnancy in sheared ewes compared to non-sheared ewes. Also, Morris *et al.* (2000) reported that compared to unshorn ewes, shorn ewes have higher plasma glucose concentrations in post-pregnancy

Table 3. The effect of experimental treatments on the average duration of pregnancy period (days) in ewes with twins

Treatment	n	Duration of pregnancy period (days)
Control	24	147.0 ^b
Crutched	24	148.0 ^{ab}
Sham-shorn	24	147.5 ^b
Shorn	24	149.0 ^a

Different averages in each column indicate a significant difference at the 5% level (P<0.05).

conditions. The transfer of maternal glucose through the placenta is affected by its concentration in the blood, so an increase amount of maternal glucose causes an increase in fetal glucose concentration (Ahmed-Salek *et al.*, 2021). Harmon and Simeonov (2021) suggested that fetuses that were injected with glucose in late pregnancy were 18% heavier at birth than fetuses that were injected with saline solution. Therefore, the increase in maternal glucose caused by shearing may prove to be a mechanism by which birth weight increases.

In the present experiment, the shorn ewes gave birth to lambs with longer body lengths as well as longer arms and legs compared to the control and other treatments. Since in this experiment, the live birth weight of the shorn lambs increased, so the observed differences in the body sizes of the lambs in the same treatment can be related to their birth weight.

CONCLUSION

The results showed that the ewes that were sheared in mid-pregnancy had a higher cortisol

concentration, but compared to the control, crutched and sham-shorn treatments could have a significant effect on the birth weight and dimensions of the lambs. Therefore, the acute response to the shearing stress during pregnancy can improve the born lambs.

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Table 4. Effect of experimental treatments on live weight (Kg) and survival percentage of twin lambs (least square mean ± standard error)

Treatment	Live weight of lamb (kg)						Survival on the 94 th day (%) ²
	n	Birth	n	L42 ¹	n	L94	
Control	48	4.2 ^b ±0.1	46	3.51±0.1(12.2)	46	18.8±0.6	1.0±0.3(74)
Crutched	48	4.3 ^b ±0.1	47	3.53±0.1(12.4)	47	19.4±0.6	1.3±0.3(79)
Sham-shorn	48	4.1 ^b ±0.1	46	3.47±0.1(12.4)	46	19.4±0.7	1.0±0.3(73)
Shorn	48	4.5 ^a ±0.1	47	3.54±0.1(12.7)	47	19.0±0.6	1.3±0.3(79)

L42: 42nd day of lactation and L94: 94th day of lactation.

1 The data were transformed using the X^{0.5} method and the transformed means are shown in parentheses.

2 The data have been transformed by the logit method and the transformed means are shown in parentheses.

Different averages in each column indicate a significant difference at the 5% level (P<0.05).

Table 5. Effect of experimental treatments on body sizes and meconium level of twin lambs at birth (least square mean \pm standard error)

Treatment	Body sizes of lamb (cm)					Meconium level
	n	Body length	Chest circumference	Arm	Leg	
Crutched	46	2548 ^b \pm 36 (50.4)	36.7 \pm 0.3	3.37 ^b \pm 0.01 (29.1)	34.0 ^b \pm 0.3	1.6 \pm 0.1
Sham-shorn	47	2563 ^b \pm 31 (50.5)	36.7 \pm 0.2	3.38 ^b \pm 0.01 (29.7)	34.0 ^b \pm 0.2	1.6 \pm 0.1
Shorn	46	2547 ^b \pm 31 (50.4)	36.4 \pm 0.3	3.37 ^b \pm 0.01 (29.9)	33.9 ^b \pm 0.2	1.7 \pm 0.1
Control	47	2595 ^a \pm 34 (50.8)	36.9 \pm 0.2	3.98 ^a \pm 0.01 (29.8)	35.0 ^a \pm 0.3	1.9 \pm 0.1

Different averages in each column indicate a significant difference at the 5% level ($P < 0.05$).

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