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ANIMAL PRODUCTION

Reproductive cycles in white Karaman ewes: comparison of ovarian hormone secretion and reproductive behavior in non-pregnant and pregnant ewes in semi-intensive conditions

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ABSTRACT. The objective of this study was to characterize the reproductive cycle both endocrinologically and behaviorally in ewes. Blood samples were analyzed by EIA for progesterone (P_4), and RIA for estradiol in order to determine the breeding strategy of non-pregnant (n = 11) and pregnant (n = 9) White Karaman throughout the year in the Middle Anatolia (39.57^{0}N, 32.53^{0}W), Turkey. Estrous cycle lengths and estrous lengths were 18.36 ± 1.03 days and 35.16 ± 5.95 hours, respectively. The breeding season durations were 283 ± 63.36 days and 229.64 ± 63.74 days according to hormonal and behavioral data respectively ($p \leq 0.01$). The highest and lowest data of estrous occurrence frequency was observed in October and June-July, respectively. P_4 levels ranged from 0.01 to 9.0 ng mL^{-1} throughout the year and from 0.01 to 0.16 ng mL^{-1} during anestrous. Mean plasma estradiol levels were 8.42 ± 2.51 pg mL^{-1} during the estrous cycle. *Postpartum* anestrous durations were 134.8 ± 18.5 and 120.78 ± 29.89 days (n = 9) according to behavioral observation and hormonal data, respectively. P_4 concentration for the months 1 – 5 of the gestation period ranged from 4.54 to 15.08 ng mL^{-1}. P_4 concentrations in 4th and 5th months of gestation were higher than the other months ($p \leq 0.01$). White Karaman reproductive activity is characterized by an extended breeding season and their relatively insensitivity to photoperiod stimulation allows for efficient out of season lamb production.

Keywords: breeding season; estradiol; indigenous ewes; *postpartum* estrous; progesterone.

Ciclo reprodutivo de ovelhas brancas Karaman: comparação da secreção de hormônio ovariano e do comportamento reprodutivo de ovelhas não gestantes e gestantes em condições semi-intensivo

RESUMO. O objetivo deste estudo foi caracterizar o ciclo reprodutivo, endocrinológico e o comportamento de ovelhas Karaman. Análises de sangue foram analisadas pelo EIA para progesterona (P_4) e RIA para estradiol para determinar as estratégias de reprodução do Karaman Branca de não gestantes (n = 11) e de gestantes (n = 9) durante o todo o ano, em Anatólia, Turkia. O Ciclo estral e a duração do estro foram 18.36 ± 1.03 dias e 35.16 ± 5.95 horas, respectivamente. As durações da época de reprodução foram de 283 ± 63.36 e 229.64 ± 63.74 dias de acordo com os dados hormonais e comportamentais, respectivamente ($p \leq 0.01$). Os maiores e menores dados de frequência do estro ocorreram em outubro e junho-julho, respectivamente. Os níveis de P_4 variaram de 0.01 para 9.0 ng ML^{-1} ao longo do ano e 0.01 – 0.16 ng mL^{-1} durante o cio. Níveis médios do plasma de estradiol foram 8.42 ± 2.51 pg mL^{-1} durante o ciclo estral. As durações do pós-parto foram de 134.8 ± 18.5 dias e 120.78 ± 29.89 dias (n = 9) de acordo com a observação do comportamento e os dados hormonais, respectivamente. Concentrações de P_4 durante os meses 1 até o 5 do período de gestação variou de 4.54 para 15.08 ng mL^{-1}. Concentrações de P_4 no quarto e quinto mês de gestação foram maiores do que nos outros meses ($p < 0.01$). A atividade reprodutiva do White Karaman é caracterizada por um período de reprodução estendido e relativa insensibilidade à estimulação do fotoperíodo permite a produção eficiente de cordeiros fora de época.

Palavras-chave: período de reprodução; estradiol; ovelhas nativas; pós-parto estro; progesterona.

Introduction

The physiological aspects of sheep breeds have led researchers to identify that reproductive activity can be controlled using assisted reproductive technologies in both male and female sheep in order to improve their reproductive management.

Hormone related factors that play a significant role in the reproductive biology of sheep breeds include: ovarian activity, estrous cycle, mating season length and the onset of *postpartum* estrous (Bartlewski, Baby, & Giffin, 2011; Basaran & Dellal, 1997; Pourlis, 2011).

In females, secretion of progesterone constantly plays a pivotal role on the effects of steroids and in the establishment of normal reproductive functions (Bartlewski et al., 2011). The regulation of the secretion and bioavailability of gonadotrophic hormones depend on a complex interaction between several internal and external factors (Rawlings & Bartlewski, 2006). Research on progesterone and estradiol 17 β concentrations in prolific and cyclic sheep breeds discovered inter-breed differences (Bartlewski et al., 1999a; Bartlewski, Beard, Cook, & Rawlings, 1998; Bartlewski, Beard, & Rawlings, 1999b; Ijabo, Jatfa, Oyedipe, Bawa, & Dawuda, 2014; Souza, Campbell, & Baird, 1997).

The sheep population of Turkey is composed by 21 native multipurpose breeds and totals 31 million head. It is estimated that 40% of the population belong to the White Karaman (WK) breed which is an indigenous fat-tailed and seasonal sheep breed (Ertuğrul et al., 2009). However, the patterns of reproductive activity for these animals have received little scientific consideration (Basaran & Dellal, 1997; Pourlis, 2011).

The objective of this study is to define optimum breeding strategy in WK by determining endocrinologically and behaviorally the reproductive cycle (estrous and anestrous) of this breed in case of pregnancy and non-pregnancy, together with the onset of the estrous period after *postpartum*.

Material and methods

Animals and location

Twenty WK ewes with the age of 2.5 years were randomly chosen from a flock of 500 animals once they had given a birth. Each animal was kept for one month before the onset of the study in order to allow for acclimatization and quarantine purposes. The mean body weight of twenty ewes was 74 ± 7.73 kg. The apron ram was 3.5 years of age (used before in mating). Eleven ewes were used for hormone analysis (1,144 data for P_4 assessment) and behavioral observation (8,030 data) without mating throughout the year. Nine ewes were mated and used for hormone analysis and behavioral observations for gestation and *postpartum* estrous intervals by using 936 and 1,935 data respectively.

The experiment was conducted in Middle Anatolia (39.57°N and, 32.53°'E) Saraykoy-Ankara-Turkey. Ankara has a hot Mediterranean/dry-summer subtropical climate that is mild with moderate seasonality. Vegetation is typically adapted to the arid summer conditions. Ewes were allowed to graze in daytime and return to their pens (200 m^2 for 20 ewes) at night in the semi-intensive conditions. During the strong winter climate, hay (*ad libitum*) and barley (200-300 gr) for each ewe were provided like small farmer conditions. The onset of estrous was detected with the aid of an apron ram for a 30-minute period, twice a day in 365 days. Nine ewes were mated and followed throughout their gestation, lactation and *postpartum* estrous period without ram effect.

Frequency of estrous occurrence (%): the number of monthly observations of estrous/monthly total number of progesterone and behavioral observations x 100.

Estrous as hormonal: progesterone concentration values below 0.5 ng mL^{-1} was accepted as estrous.

Estrous as behaviorally: the ewe was accepted as being in estrous by standing still and allowing the ram to mount to her and displaying ram-seeking behavior or showing the lordosis reflex.

Estrous cycle lengths (days): extending from one period of estrous to the next, accompanied by behavioral changes and hormone levels.

Estrous lengths (hours): the period from the onset of estrous until the end of estrous.

Anestrous length (days): the period from the end of estrous cycle to the onset of estrous.

Breeding season (days): 365 days – anestrous length.

Four seasons were defined; 1. First season: June 21th (the longest day) - September 21th; 2. Second season: September 21th – December 21th (The shortest day); 3. Third season: December 21th – March 21; 4. Fourth season: March 21 - June 21th.

Serum progesterone (P_4) and estradiol 17 β (E_2) analysis

Blood samples were collected twice a week throughout the 12 month via a jugular vena puncture. Serum was separated by centrifugation in +4°C, 3,000 rpm, 15 minutes, divided into two parallel for one portion and stored at -20°C until Enzyme Immunoassay for P_4, Radio Immunoassay for estradiol analysis.

Blood samples were analyzed by Enzyme immunoassay for P_4 (Van de Wiel, Koops, & Vos, 1986). Progesterone labeled with Horse Radish Peroxides and anti-progesterone–IgG produced in Nuclear Biotechnology Research laboratory of Turkish Atomic Energy. Sensitivity of the test was

1.25 pg mL^{-1}. The intra and inter assay coefficient variation of the test was 8.63 and 11.6%, respectively. Solvent (methanol) extractions were performed on serum samples for estradiol 17β analysis. Concentration of E_2 was determined by solid phase 125 I RIA method (ICN 17β-Estradiol 125 RIA Kit: ICN Pharmaceuticals, Inc. Diagnostics Division, Costa Mesa, California) in two cycles for 11 ewes (in duplicate aliquots for each sample). Assay methodology was described by Dieleman and Bevers (1987). Sensitivity of the test was 10 pg mL^{-1}. The intra and inter assay coefficient variation of the test was 9.6 and 10.95 %, respectively.

Statistical analysis

Chi square test was performed to investigate the statistical significance of categorical variables such as; occurrence and frequency of estrous detected by both hormonal and behavioral observations. For continuous data; such as plasma progesterone concentration; General linear model was performed to test the statistical significance between multiple groups such as season and months. In case of significance, Turkey post hoc test was applied for investigating pair wise differences. Inter-rater agreement statistic (Kappa) was used to evaluate the agreement between results of behavioral and hormonal parameters. Student t test was used to compare the days of breeding and anestrous seasons between hormonal and behavioral estrous observational categories. Data were represented as means ± SD in figures and text. For all statistical analyses a p value of ≤ 0.05 was considered significant.

Results

Behavioral observations for the lengths of estrous cycle and estrous

The overall mean of estrous cycle lengths (days) of 11 sheep was 18.36 ± 1.03, ranging from 16 to 20.5 days and estrous length was 35.13 ± 5.95 hours, ranging from 26.4 ± 10.0 to 47.25 ± 14.1 hours. One extreme value regarding the estrous cycle length (34 – 35 days) was observed in one animal during the transition to anestrous period and this was eliminated from the analysis.

Breeding season and anestrous lengths

The means of anestrous lengths (days) of 11 sheep obtained by hormonal measurements and behavioral observations were 81.81 ± 63.36 and 135.36 ± 63.49, respectively. Breeding seasonal lengths were also obtained from hormonal and behavioral analysis and the mean values were 283 ± 63.36 and 229.64 ± 63.70, respectively. Significant differences were found among hormonal and behavioral data (p ≤ 0.01) for both breeding season and anestrous length (Figure1a and b, respectively). According to progesterone levels, *corpus luteum* was active in 72.7% of the animals during the transition period to anestrous season.

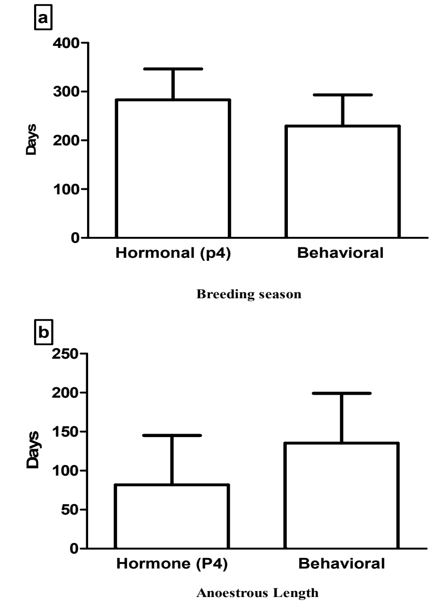

Figure 1. Breeding season length (a) and anestrous lengths (b). In both graphics there was a significant difference (p ≤ 0.01) between hormonal and behavioral assessment.

Distribution of progesterone concentration throughout the year

Progesterone level range in estrous cycle varied from 0.01 to 9 ng mL^{-1}; while the overall mean value of progesterone in an estrous was 0.01 ± 0.16 ng mL^{-1} in ewes (n = 11).

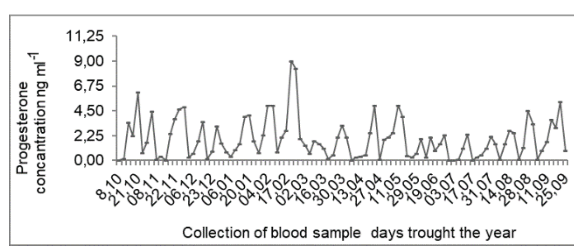

Figure2. An example of estrous cycle of an ewe without anestrous period with progesterone profile throughout the year.

Progesterone levels of estrous cycles were between 0.02 - 6.17 ng mL^{-1}, in an example ewe (Figure 2). Hormonal activity continued throughout the year without anestrous.

Estradiol 17β profile during the estrous cycle

Estradiol 17β analysis showed that the highest level of the overall means of estradiol (8.42 ± 2.51

pg mL^{-1}) was accepted the onset of estrous (Figure 3). Estradiol concentration decreased towards day 8 of the cycle and increased during diestrous (day 11; 6.09 ± 2.87 pg mL^{-1} a small peak thought to be non-ovulatory waves). It decreased to its minimum level at the end of the cycle (day 15; 3.50 ± 1.50 pg mL^{-1}).

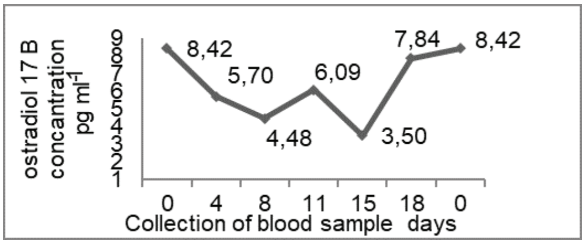

Figure3.The overall mean values of estradiol 17β obtained from the three estrous cycles of 11 ewes. 0 = estrous.

Progesterone concentration in estrous cycle

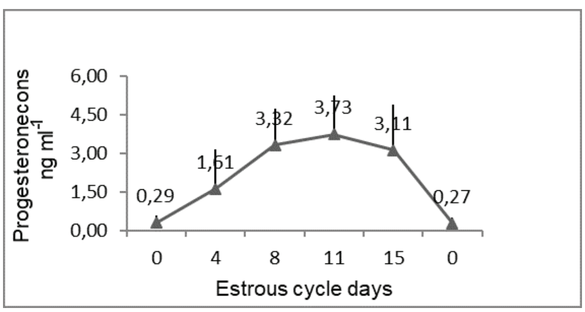

Figure 4. The overall mean values of progesterone obtained from the three estrous cycles of 11 ewes. 0 = estrous.

Mean progesterone concentrations were 0.30±0.29 ng mL^{-1} in estrous, which rise slowly towards to metestrous. After day 4, there was an accelerated rise to diestrous, with maximum levels reached on day 11 (mean 3.73±1.50 ng mL^{-1}). Between day 11and 15, progesterone levels remained high. After day 15, it fell suddenly to a basal level in prestrous (Figure 4).

Frequency of estrous occurrence within a year

Estrous was observed in every month of the year in White Karaman sheep. The highest frequency of estrous occurrence was seen during October in terms of P4 level and this level remained from August to March. The lowest frequency levels occurred in June and July. Monthly and seasonal comparison showed that there were differences ($p \leq 0.01$) in frequency of estrous occurrence determined by P4 levels (Figure 5).

Monthly and seasonal comparison also show that there were differences ($p \leq 0.01$) in frequency of estrous occurrence determined by behavioral assessment.

With shortening daylight hours, frequency of estrous occurrence in June (10.4%) began to increase during September (28.6%) and reached its maximum level (36.4%) in October. Levels gradually decreased until May where they reached similar levels observed in June, except an increase was seen in January.

In the months from August to February, the frequency of estrous occurrence levels provided by P_4 measurement and behavioral observations matched; while the results determined by P_4 measurement were higher than that of behavioral observation from February to the end of July. In spite of this difference, frequency of estrous occurrence was verified by Kappa test ($p \leq 0.01$) considering the results of P_4 level and behavioral observation.

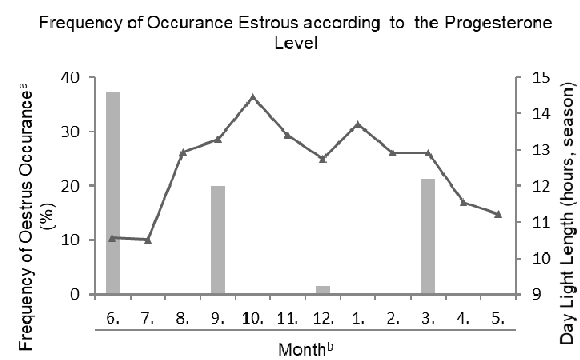

Figure 5. Line shows monthly and seasonal estrous frequency according to the P_4 levels. Columns show day light (four season as describe) hours (n = 11). $^{a,b}\chi^2$ ($p \leq 0.01$).

Gestation and post-partum periods

Table 1. Gestation and birth parameters of White Karaman Sheep.

White Karaman	Gestation Period	Litter Size	Twins rate, %	Birth weight, kg
n = 9	148.56 ± 0.88	1.56	55	4.0±0.85

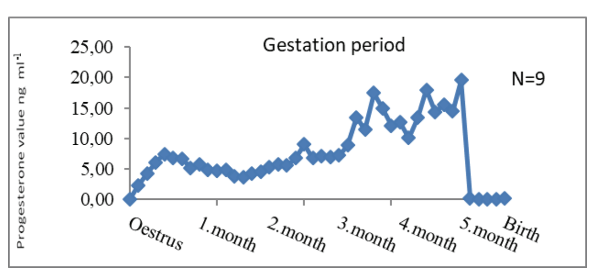

Figure 6. The mean value of Progesterone concentrations in gestation period of White Karaman Sheep (n = 9).

Plasma progesterone concentration for 1, 2, 3, 4 and 5th months of gestation were 5.61 ± 3.46, 4.54 ± 0.53, 6.93 ± 1.05, 13.03 ± 2.70, 15.08 ± 3.09 ng mL^{-1}, respectively (Figure 6). Progesterone concentrations in the 4th and 5th months were higher than those in the other months ($p \leq 0.01$).

The births were started to occur in the middle of the March. *Postpartum* estrous intervals were 134.8 ± 18.5 days and 120.78 ± 29.89 days (n = 9) according to behavioral observation and hormonal data including 2 months lactation period, respectively

Discussion

The estrous cycle length in White Karaman ewes obtained throughout the year resembles that of other fat tailed sheep breeds (Ali, Derar, & Hussein, 2006). Results from previous research in White Karaman sheep (Kaymakçı, Aşkın, & Karaca, 1987) showed slightly shorter estrous cycle lengths. The difference can be explained by the method used in that work depends on only behavioral observations. The studies about estrous cycle lengths showed that they looked similar for most other sheep breeds (Ali et al., 2006; Bartlewski et al., 1999a; Bartlewski et al., 1999b; Evans, Duffy, Hynes, & Boland, 2000; Sulu, Özsar, Güven, & Bağcı, 1993). However, year, season, individual and environmental factors can influence the cycle length in the same breed, although these differences are relatively small, usually not exceeding 1-day (Goodman, 1994). Estrous lengths obtained from behavioral observations made at 12 hours' intervals were similar to others (Dağlıç, Awasi, Menemen and Karacabey merino) in Turkey (Kaymakçı, 1984). Similar results were also reported from Awasi and Tabasco sheep in Israel (Sefidbakht, Mostafavi, & Farid, 1978).

Breeding season lengths according to endocrinological and behavioral data in WK were found noticeably longer than breeding season lengths for Ile de France and Gentile de Puglia breeds (Dell'Aquila, Varriale, & Alberico, 1988). White Karaman breeding season lengths from behavioral data were also longer than some Turkish sheep breed such as Dağlıç and Chios (Kaymakçı, 1984; Kaymakçı et al., 1987). These results confirm that White Karaman sheep has a relatively long mating season and short anestrous period compared to other sheep breeds in Turkey and also in the mid-latitude countries (35-40° N). The results regarding anestrous length in WK were similar to other sheep breeds from mid-latitudes (35-40° N), such as the Australian Merino, other Mediterranean breeds (Gómez-Brunet, Santiago-Moreno, Toledano-Diaz, & López-Sebastián, 2012) and Spanish Manchega ewes (Gómez-Brunet et al., 2008). WK, like some sheep breeds present at the same latitude showed reduced seasonality, displaying a long breeding season (summer to winter) and a short anestrous period. In this study, cycling rate was relatively high throughout the year which is in agreements with the results of high cycling rate in the sheep of similar latitudes (Gómez-Brunet et al., 2012).

At the end of breeding season, hormonal parameters showing estrous were not proved by behavioral observations in some ewes. This indicates the presence of behavioral silent estrous. This was not observed in Fin ewes (prolific breed) but was found in Merino ewes during the same period (Wheeler & Land, 1977). P4 analysis, 7 out of 11 animals showed the general pattern with regular peaks of hormone followed by a short period of anestrous while 4 out of them had a regular cycle throughout the year without the anestrous. It can be said that 36.4% of ewes show estrous regularly throughout the year. These results showed that WK has a comparatively long breeding season when compared with other northern hemisphere sheep breeds (Bartlewski et al., 1999a; Dell'Aquila et al., 1988; Gómez-Brunet et al., 2008). The results from our anestrous P4 levels were also similar with anestrous P4 levels from other sheep breed (Ferreira-Silva et al., 2017; Sasa, Rodrigues, Nonaka, Balieiro, & Coelho, 2016).

The P4 values measured in estrous cycle of WK were not found to be different from the other sheep breeds such as Western White-Faced ewes and native Chios, Welsh Mountain (Bartlewski et al., 1999b; Shackell et al., 1993; Sulu et al., 1993) with the exception of Finn ewes. The difference of max P4 values in Finn ewes can be explained by their prolific characteristics of their estrous cycle (Bartlewski et al., 1999a). The regulation of the secretion and bioavailability of gonodotrophic hormones depend on complex interaction among internal and external factors (Rawlings & Bartlewski, 2006), also breed and inter breed interactions are so important (Goodman, 1994; Mukasa-Mugerwa, Ezaz, & Viviani, 1990). Consequently, non-prolific breeds of sheep may produce more progesterone during metestrous and diestrous compared with prolific animals (Bartlewski et al., 1999b). P4 levels in the estrous cycle in WK show that it is a non-prolific breed (Basaran & Dellal, 1997) like White-faced ewes.

There are 3-4 major increases in circulating concentrations of estradiol during the estrous cycle in ewes. In non-prolific sheep breeds, the amplitude of estradiol fluctuations associated with all non-ovulatory waves during diestrous does not differ from that during the pre-ovulatory rise in estradiol secretion. Peak values for estradiol concentration during the pre-ovulatory period are typically higher than those in the luteal phase of the estrous cycle in prolific genotypes. On days 1-5, days 14-15 and day 17 of the cycle, the mean serum concentrations of estradiol were significantly higher in Finn sheep when compared with White-Faced ewes (Bartlewski et al., 1999a; Evans, 2003). In WK, the maximum value of estradiol was obtained on day 0-2 and the second one on the day of 11. This was also similar to levels found in White Face ewes.

The annual rhythm of ovarian cyclicity is characterized by season-dependent cessation (anestrous) and restoration (breeding season) of ovarian ovulatory cycles. Ewes are seasonally polyestrous animals with normal ovulatory cycles occurring, in most northern hemisphere breed during the fall and winter months (Ali et al., 2006; Bartlewski et al., 2011; Hayder & Ali, 2008). This is also the case for WK with an extended breeding season which continues from August to April.

In *postpartum* period, the onset of ovarian activity detected through plasma P4 concentrations was earlier than the behavioral estrous signals (Hayder & Ali, 2008). Most ewes had several silent ovulations before their first detected estrous. The *postpartum* estrous interval was a slightly shorter than the Rasa Aragonesa ewes and the reason for this difference can be explained by using ram effect in that study (Abecia et al., 2017). The onset of natural breeding season is affected by the lambing season (Forcada et al., 2006). The births in many Anatolian breeds start in December-January. Although it was March for the beginning of birth in this study with a relative short *postpartum* estrous.

Conclusion

To our knowledge, this is going to be the first published study on the reproductive cycle patterns of WK and provides novel information on behavioral and endocrine response. The results show that WK ewes have an extended breeding season, short *post-partum* estrous interval and active ovarian activity compatible with estrous behavioral activity except two months under their natural environmental conditions. WK ewes due to their genetic selection are less sensitive to photoperiod changes. This allows for the manipulation of estrous cycle through the use of exogenous hormones, bio-stimulation techniques and natural methods leading to a more efficient out season lamb production.

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