



THE EFFECT OF SALT-FREE - SALT DIET ON THE REPRODUCTIVE PERFORMANCE OF ILE DE FRANCE EWES

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Summary

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The aim of the present study was to establish the effect of salt-free - salt diet (SFSD) on the size of antral follicles during salt consumption, the duration of controlled breeding campaign and the fertilities of ewes from the Ile de France breed. The experiment was carried out with 57 ewes, which were divided into 3 groups (19 ewes in each) depending on whether they were subjected to SFSD and their contacts with rams during the first 8 days of the beginning of breeding: Group I – SFSD + ram contact; Group II – only SFSD; Group III – no SFSD, only ram contacts. The first day of the salt diet coincided with the first day of ram contacts. Transrectal ultrasound examinations of the ovaries were done on days 1, 2, 3, 5, 6 and 7. The time of manifestation of estrus (in days), pregnancy rate and fecundity were studied. A significant effect of time ($P < 0.05$) and diet ($P < 0.01$) on the size of follicles was established. The onset of the first estrus, the shortest terms of breeding campaign, the fertility and the fecundity results gave us reason to favour the scheme applied to Group II. A stimulatory effect of the diet in that study was demonstrated, but the pattern was different from our previous studies.

Key words: estrus, ewes, fertility, follicles, salt

INTRODUCTION

The synchronisation of fertility and lambing of ewes is one of the main elements in the reproductive management in sheep breeding. The methods of estrus synchronisation can be classified as natural (non-hormonal) and pharmacological (hormonal). Non-pharmacological methods are alternative to the conventional estrus synchronisation, an important issue in today's consumer attitudes (Martin *et al.*, 2004). The most popular non-pharmacological

methods are: the ram effect, flushing, salt-free-salt diet (SFSD), vitamin and mineral supplementation (Tyankov *et al.*, 2000).

The introduction of ram(s) (i.e. ram effect) to isolated anoestrus ewes leads to increase of pulsatile secretion of LH, which may end with LH surge followed by ovulation (Knight *et al.*, 1978; Oldham & Cognié, 1980; Martin *et al.*, 1983; Ungerfeld *et al.*, 2004). The ram effect can be achieved without prior isolation of ewes

from rams (Cusha *et al.*, 1992). It is applicable in breeding season too, as the introduction of rams to cyclic ewes stimulates an increase in pulsatile LH secretion, independent of ewe genotype or stage of the estrous cycle (Hawken *et al.*, 2007). Ewes generally ovulate in response to ram introduction within 54 h (Oldham *et al.*, 1978), but the first ovulation following introduction of rams is usually not accompanied by behavioural estrus (Oldham & Cognié, 1980; Nugent *et al.*, 1988). In some ewes there is an initial short luteal phase of 4–5 days, then a second ovulation without any signs of estrus, followed by a luteal phase of normal duration (Ungerfeld *et al.*, 2004). These differences of ovarian response at ewes could lead to two surges of manifesting synchronised estrus induced by the ram effect – the first is between days 17–20 and the second is between days 21–25 after ram introduction (Martin *et al.*, 1986).

The method of salt-free - salt diet has been traditionally used in Bulgaria at the beginning of breeding season. The traditional use of the SFSD comprises starting with salt-free diet for 6 or 7 days, followed by salt diet of the same duration with a daily dose of salt (NaCl) 15–20 g per ewe (Solomonov, 1977; Tsolov *et al.*, 2000; Hristova, 2007). The effect of SFSD is 40–50% of ewes coming in estrus during the first week from the start of the breeding campaign (Tyankov *et al.*, 2000). For the last 10 years, series of experiments were done with the aim to test the effect of the diet or the diet as a part of estrus synchronisation protocols. The obtained results were contradictory and up to now there are not convincing grounds accept or reject the SFSD (Metodiev, 2013). Nedelkov & Todorov (2012) and Nedelkov *et al.* (2012) investigated the effect of salt-free - salt diet and concluded

there it had no effect, whereas Hristova (2007), Metodiev *et al.*, (2007; 2009; 2010), Metodiev (2014) reported satisfying results in line with data reported by Tyankov *et al.* (2000).

The aim of the present study was to establish the effect of salt-free - salt diet on the size of antral follicles during salt consumption, the duration of controlled breeding campaign and the fertility of ewes from the Ile de France breed.

MATERIALS AND METHODS

The experiment was carried out in March (second half), April, May (first half) of 2014, with 57 clinically healthy Ile de France ewes (aged 3–4 years), raised in the experimental base of the Institute of Animal Science – Kostinbrod. Ewes were with BCS of 3.0–3.5 and live weight between 70–80 kg. All ewes had normal previous lambing (lambled during October 2013) and lambs were weaned at mid-January 2014. In previous works of ours (Metodiev *et al.*, 2010; Metodiev & Raicheva, 2011) and also our field experience since 2005 with Ile de France ewes from the flock of IAS-Kostinbrod, we successfully fertilised them without hormonal stimulation by that time of the year (spring, April-May). So the experiment was designed to start in the beginning of the breeding season.

Ewes were fed (per ewe): meadow hay 0.7 kg, corn silage – 1.5–2.0 kg and concentrate (0.150 kg dried distiller's grain solubles and 0.150 kg wheat grain). After the second half of April (grazing on pasture about 2–3 hours per day), the feeding with hay was stopped, but the feeding with corn silage and concentrate was the same. The salt diet, preceded by 10 days of salt-free diet (which means that the whole salt-licks were removed and the ewes didn't

consume any salt), continued with 7 days during which the salt licks were returned and salt was added in the concentrate at a daily dose of 20 g per ewe. The ewes were divided into 3 groups (19 ewes in each) according to whether they were subjected to SFSD and their contacts with rams during the first 8 days of the beginning of breeding: Group I – SFSD + ram contact; Group II – only SFSD; Group III – no SFSD, only ram contacts. The first day of the salt diet coincided with the first day of ram contacts (also the start of the breeding campaign (BC).

Transrectal ultrasound examinations of the ovaries were done on randomly selected 15 ewes (5 ewes from each group) on days 1, 2, 3, 5, 6 and 7 of the salt diet. Follicles with diameter ≥ 3.0 mm and corpora lutea were counted and measured. Day 4 was skipped due to technical reasons. The ultrasound screenings were performed in the morning, after that the ewes consumed salt, mixed with wheat grains and DDGS (Group III did not receive salt with the mix). Ultrasound examinations were conducted in standing position of the ewes. First the ampulla recti was evacuated from faeces, after that the ultrasound probe, covered with gel, was inserted with the help of a cradle into the rectum. The equipment included a digital portable ultrasound system ALOKA ProSound 2 (Aloka Co., Ltd.) supplied with electronic linear transducer UST 5820, with frequency of 7.5 MHz. After concentrate intake, teasers were introduced to ewes (1 teaser to 20 ewes). After the 8th day all ewes were mixed with ewes from the flock and the ewes in estrus were detected by teasers once daily in the morning for 1–1.5 hour (1 teaser for 50 ewes). Ewes from Group II were put in contact with rams on day 9 after the beginning of the experiment. Experimental ewes in heat

were inseminated naturally, once in the middle of the day, when they manifested estrus. Six clinically healthy rams at the age between 1.5 and 5 years were used, one ram performing maximum 3 servings per day.

The following reproductive traits were studied: the time of manifestation of estrus (in days), pregnancy rate and fecundity.

Breeding and estrus observation by teasers for Group I and Group III started at Day 1 of the salt diet. The first day of the salt diet coincided with the first day, in which ewes contacted the rams (for Group I and Group III). Breeding and estrus observation by teasers for Group II started at the second day after the end of the salt diet e.g. day 9 of the breeding of Group I and Group III. For the first 8 days they were tested for presence of heat with an estrus detector (Draminski Ltd). All ewes that had electrical resistance ≤ 350 units were considered to be in estrus (according to user's manual and our field observations with ewes in estrus, previously detected by teasers). The experiment lasted till all experimental ewes having manifested estrus for the first time.

Fertility (at first estrus) was defined as the ratio of the number of pregnant ewes to the number of ewes in the group. Fecundity was defined as the number of lambs born from pregnant ewes (including all born lambs – live born, dead born and aborted).

A linear mixed model was developed in which the dependent variable was the size of follicles, independent variables were the group and time. The random time effect and intercept were included. The fixed effect was associated with the group and time. The restricted maximum likelihood method for the analysis of data is used. One-way ANOVA and post hoc LSD analysis were used to compare the

Table 1. Ovarian structures (mean±SEM) in the three groups of sheep (I: salt-free-salt diet + ram contact; II: salt-free-salt diet only; III: ram contact only) according to the time of examination

Time	Groups	Ovarian structures			
		Follicles, cm		Corpora lutea, cm	
		n	size, cm	n	size, cm
Day 1	SFSD+ram contact	14	0.51±0.09	2	0.45±0.05
	SFSD	9	0.59±0.21*	1	0.5
	Ram contact	17	0.44±0.09 ^A	0	
Day 2	SFSD+ram contact	14	0.52±0.11	2	0.47±0.05
	SFSD	12	0.57±0.25	1	0.53
	Ram contact	16	0.60±0.12 ^B	0	
Day 3	SFSD+ram contact	13	0.58±0.15	3	0.43±0.05
	SFSD	11	0.69±0.20	0	
	Ram contact	9	0.56±0.11	3	0.43±0.02
Day 5	SFSD+ram contact	12	0.49±0.16	2	0.36±0.02
	SFSD	14	0.56±0.18	5	0.45±0.02
	Ram contact	16	0.52±0.15	5	0.41±0.03
Day 6	SFSD+ram contact	10	0.60±0.11	3	0.48±0.04
	SFSD	13	0.65±0.21	5	0.47±0.02
	Ram contact	15	0.57±0.10	4	0.54±0.05
Day 7	SFSD+ram contact	14	0.58±0.10	3	0.46±0.03
	SFSD	14	0.58±0.13	3	0.51±0.05
	Ram contact	17	0.58±0.13	3	0.48±0.01

* P<0.01 between Group II and Group III; ^A statistically significant differences between the follicle size at day 1 and day 2 (P<0.001); day 3 (P<0.05); day 6 (P<0.01); day 7 (P<0.01); ^B statistically significant differences between follicle size at day 2 and day 5 (P<0.05).

size of follicles. All data were calculated by IBM SPSS 19.

RESULTS

A significant effect of time (F=4.273, P<0.05) and diet (F=3.393, P<0.01) on the size of follicles was established. Significant differences for the size of follicles were established only between Group II and III for day 1 (P<0.01, F=3.895) (Table 1). The time had significant effect only on size of follicles of Group III (P<0.01, F=3.915) (Table 1). For the studied period, ewes from Group II had follicles with the largest size – between 0.58 and 0.69 cm and also follicles with the maximum size – up to 1.2 cm.

Most of the ewes came in estrus for the first time between days 15–27 of the beginning of breeding campaign (Group I – 12/19, Group II – 13/19, Group III – 13/19). It should be noticed that the breeding campaign in Group II started 8 days later (on day 9) and this group had the shorter bounds of breeding – 23 days (Fig. 1).

The test with an estrus detector showed that for the first 8 days 8 ewes had signs of estrus without ram contacts. Five out of eight ewes came in estrus again in the interval 16–18 days, detected by teasers (these were days 18–21 of the beginning of breeding campaign).

The fertility rates were high for all groups, but Group II had a fertility of

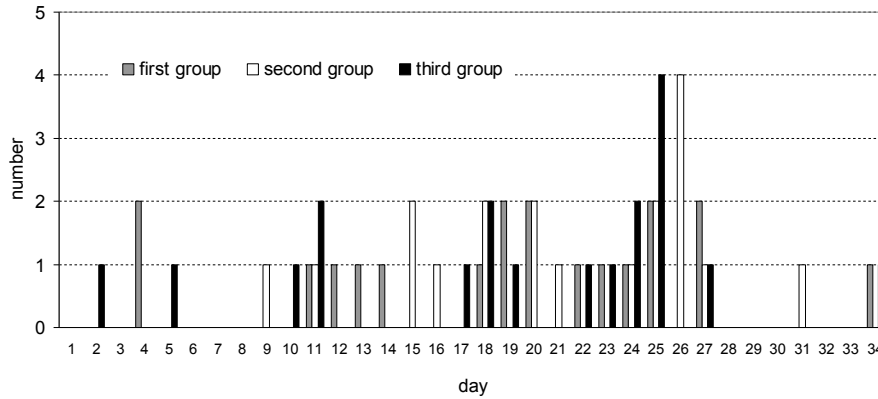


Fig. 1. Onset of first estrus in ewes detected by a teaser from the three experimental groups (first group: salt-free-salt diet + ram contact; second group: salt-free-salt diet only; third group: ram contact only).

Table 2. Fertility and fecundity of the three experimental groups of ewes (first group: salt-free-salt diet + ram contact; second group: salt-free-salt diet only; third group: ram contact only)

Groups	Fertility (at first estrus)		Fecundity	
	n	%	n	%
Group I	16/19	84.21	24	150.00
Group II	19/19	100.00	31	163.16
Group III	16/19	84.21	25	154.25

100% (every ewe conceived at first estrus, Table 2). The fecundity was similar among the groups, with the highest value in Group II – 163.16% (Table 2).

DISCUSSION

Significant differences in the size of follicles were established only between Group II and III for day 1 ($P < 0.01$, $F = 3.895$; Table 2). We suppose that this may be due to a salt deficiency during the previous 10 days. It is acknowledged that the deficiency in sodium and associated excess of potassium can reduce fertility by irregular estrus cycles, endometritis and follicular cysts (Moinier & Drüeke, 2008).

The significant effect of time on size of follicles for Group III ($P < 0.01$, $F = 3.915$) was expected and corresponded to literature data for the pattern of follicle waves (Ginther *et al.*, 1995; Bartlewski *et al.*, 1998; 1999; Evans *et al.*, 2000) and the data about the ram effect (Ungerfeld, 2003).

The results concerning follicle size of the other two groups were interesting. Both groups (I and II) during the whole studied salt period had follicles with a size near ovulation. Group II had follicles with the largest size – between 0.58 and 0.69 cm and also follicles with the maximum size – up to 1.2 cm. These results meant that these follicles didn't ovulate and persisted. We could not tell that these folli-

cles were cysts, because cysts are defined as large thin-walled follicular structures that do not ovulate but persist for at least 10 days in the absence of a corpus luteum (Kesler & Garverick, 1982). In our case both groups had corpora lutea, so maybe there was no LH deficiency. Also after finishing the SFSD, the ewes showed normal cyclicality.

The pathophysiology underlying follicular cysts appears to be lack of an estradiol (E_2)-induced GnRH/LH surge due to hypothalamic insensitivity to E_2 (Gümen & Wiltbank, 2005). There were ewes from Group II in estrus, so they should have high estradiol activity. On the other hand, ewes from Group I were in contact with rams, so they should have high LH levels. Due to both reasons we have hypothesised (Metodiev *et al.*, 2009) that there was a synergic effect between ram effect (high LH release) and the salt-free - salt diet.

In our previous studies (Metodiev *et al.*, 2009; 2010) we suggested that the synergic effect between the ram effect and SFSD leads to estrous behaviour in more than half of ewes from the studied groups. Ewes from Group I however didn't show this pattern. We supposed that this may be due to several reasons – behaviour of rams, breed, time of the year. The rams were with low levels of sexual performance (without enough sexual activity, in more cases the ewes first started a contact with the rams), hence they didn't induce a sufficient LH surge. It is known that rams that exhibit high compared to low levels of sexual performance (on the basis of serving capacity tests) induced a greater percentage of ewe's ovulations (Perkins & Fitzgerald, 1994). Also, previous study with Ile de France and SPBM rams raised in IAS – Kostinbrod, showed that the former were in the intermediate group

compared to the latter breed of rams, distinguished with high serving capacity (Kistanova *et al.*, 2005).

Another reason was the weather. It may be a factor, because our previous studies were done during warmer months – May, June, July and August, whereas the present study was conducted in March and April. We obtained better results by using this protocol (during July and August) with SPBM ewes (Metodiev *et al.*, 2009; Metodiev, 2014). The above mentioned considerations (behaviour of rams, breed, time of the year) should be taken into account in next studies.

It was surprising that the first estrus, terms of breeding campaign, pregnancy rate, and fecundity were the best in Group II. The shortest term of breeding campaign gave us reason to favour the scheme applied to Group II. Fertility and fecundity rates were similar in both groups. It is well known that after natural estrus (not induced by hormonal treatment) and natural matings, the fertility rates were the best.

Salinity is an increasing problem in agriculture worldwide (Ghassemi *et al.*, 1995). One of the available options to revegetate salinised landscapes and re-establish grazing systems, is the use of halophytic plants such as saltbush (Masters *et al.*, 2007). In terms of integrating saltbush into a productive system, Digby *et al.* (2008) showed that high salt intake did not decrease the reproductive capacity of the ewe. Personal communications with farmers in Bulgaria revealed that many practice so called „provender“ that means adding a high dose of salt in the concentrate once a week, especially when ewes graze on highland pastures or before preparing them for breeding.

We agree with the statement that the salt-free - salt diet acts as a stimulus on

reproductive system (Bankov *et al.*, 1989). Aristotle was perhaps the first to mention that sheep were in a better condition by keeping their hydro-mineral balance under control and said that the animals that drink saline water can copulate earlier (Moinier & Drüeke, 2008). We demonstrated a stimulatory effect in our study, but its pattern was different from previous studies of ours. More experiments should be conducted to understand the mechanisms of that effect.

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