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THE POSSIBILITY FOR OESTRUS SYNCHRONIZATION BY SALT-FREE-SALT DIET IN SOME SHEEP BREEDS REARED IN BULGARIA

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Abstract

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The purpose of these studies was to determine the effectiveness of the salt-free-salt diet, which is widely applied in Bulgaria as a non-hormonal method for oestrus synchronization of ewes. The main point of this diet consists of complete deprivation from salt in ewe's diet for approximately two weeks followed by the addition of 15-20 g dietary salt per ewe per day for 5-7 days. Three group experiments have been carried out. The first experiment was conducted with Tsigai ewes, the second – with Karakachan Sheep, and the third group experiment has been carried out with ewes of the Bulgarian Dairy Synthetic Population. The animals of same breed were divided into two groups equalized by age and BCS. Both groups were placed at the same conditions of feeding and keeping. Control groups received regularly salt, while experimental groups was subject to the salt-free-salt diet. According to literature data a few days after supplementing ewes with a high dose of salt, almost 50% of ewes came in oestrus. In our experiments tracking of oestrus behavior and insemination of ewes started on the third day from the introduction of high salt doses. From the 3rd to the 12th day after giving salt, the following percentages of ewes from the different breed came in oestrus: Tsigai breed – 15.7% from 134 ewes in the control group and 13.5% from 133 ewes subject to the salt-free- salt diet; Karakachan Sheep breed – 16.4% from 55 ewes in the control group and 25,9% from 54 ewes subject to the salt-free-salt; Bulgarian Dairy Synthetic Population Sheep – 3.2% from 154 ewes in the control group and 3.8% from 159 ewes subject to the salt-free- salt diet. Total for all sheep breeds, from 343 ewes in the control groups at oestrus were 10.2% and from 346 ewes subject to the salt-free- salt diet – 11.0%. Three, 10 and 17days after inclusion of 20 g common salt in diet one out of five ewes from the control group had increased blood progesterone concentration up to 1.796 ng/ml. In the group fed the salt-free-salt diet the progesterone values were bellow 0.841 ng/ml in all ewes. The ultrasonographic examinations performed in June, coinciding with the late anoestrous period, showed a corpus luteum in ewe with increased values of progesterone. In the beginning of the breeding season at the 10th day after giving 20 g salt, 9 out of 20 ewes subject to the salt-free- salt diet formed a corpus luteum vs. 7 out of 20 ewes in the control group. Group experiments, assays of serum progesterone and ultrasonographic examinations revealed no significant influence of high amounts of dietary salt on the onset of oestrus from the 3rd to 12th days after the supplementation. In conclusion, there is not significant effect of salt-free-salt diet on in provoking induction of oestrus in ewes.

Key words: oestrus synchronization, salt-free-salt diet, ewes, breed, progesterone, ultrasonography

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Introduction

According to Bulgarian university textbooks (Stankov, 2000; Slavov, 2005; Nikolov, 2008), monographs (Solomonov, 1977; Bankov et al., 1989; Georgiev, 1996) and popular scientific literature (Doichev et al., 1976; Solomonov and Jeliazkov, 1976 and others) the salt-free-salt diet stimulates the onset of oestrus in most ewes a few days after its application. This diet includes a complete salt deprivation of ewes for approximately two weeks, followed by inclusion of 15-20 g of salt for 5-7 days. The published experimental data are not conclusive because of the combination of salt-free-salt diet with other factors (Bratanov et al., 1975; Doichev et al., 1976; Solomonov and Jeliazkov, 1976; Solomonov, 1977; Jankov et al., 2004; Metodiev et al., 2007; Hristova, 2007), a lack of control group (Metodiev et al., 2009) or non-significant differences (Metodiev et al., 2010). In our previous experiments the synchronizing effect of the salt-free-salt diet has not been confirmed.

Moinier and Drueke (2008) published an extensive review on concepts for the positive role of dietary salt on health and reproduction in humans and various animal species from Greek antiquity to the present days. The authors pointed out that Aristotle had recommended the use of saline water for ewes to be mated earlier. However, none of the allegations in this article about the positive influence of salt on reproduction was not supported by reliable scientific evidence.

In the international scientific literature a considerable number of studies for the influence of rations with a high sodium chloride content in feeding of ewes have been published (Wilson, 1966; Wilson and Hindley, 1968; Meintjes and Olivier, 1992; Digby et al., 2007; Blache et al., 2007; Blache et al., 2008), but a positive influence on provoking and synchronization of oestrus has not been noted.

For complete clarification of controversial data about the effect of the salt-free-salt diet on oestrus synchronization of ewes and the conditions in which such an effect occurs, additional studies are necessary. This gives us a reason for conducting the present experiments.

The purpose of these studies was to examine the influence of a salt-free-salt diet on oestrus synchronization in four sheep breeds reared in Bulgaria - Tsigai, Karakachan Sheep, Bulgarian Dairy Synthetic Population and Pleven Blackhead.

Materials and Methods

First group experiment

The experiment was carried out in 2011 and 2012 at the private farm in Momchilovtsi village, Smolyan region. The ewes from one of the flocks in the farm were used. The flock included 267 Tsigai ewes. The flock included twenty six maiden ewes 1.5 years of age and the other 2.5 to 6.5-year-old ewes.

Ewes were assigned to two groups at 20.07.2011. The groups alloted by age had an equal body condition score measured at the beginning of the experiment (Table 1).

The first group served as a control (n=134) and received regularly sodium chloride according to adopted practices in the farm by providing rock *ad libitum*.

The second (experimental) group (n=133) was fully deprived of sodium chloride for 15 days (from 20.07 to 3.08.2011), then the fed concentrate (wheat bran) was supplemented with fine table salt at a daily dose of 20 g per ewe for 5 days. The period of giving high doses of salt included two days before insemination and three days after that.

Control and treatment groups were gathered in one flock, 20 days after their separation for offering the salt-free-salt diet in the experimental group. After the reunion of the two groups, rock salt was available for all ewes.

Except for sodium chloride, both groups of ewes received exactly the same ration which consisted of 200 g wheat bran per ewe daily. Grazing and farming conditions of the two groups, reared in one flock by the same shepherd, were also identical.

In both groups of ewes the insemination started at the same time, two days after the start of supplementing the experimental group's ration with 20 g salt per ewe daily. The insemination of ewes in oestrus was performed naturally by the hand service mating system according to the breeding plan. The ewes in oestrus were detected by teaser rams equipped with aprons (cloth covering the abdomen tied at the withers and the

croup) to prevent breeding. Teasers were placed in the flock in the morning and evening for 45-60 min. each time.

All rams were separated from ewes and reared separately for 52 days before the beginning of breeding period.

For the insemination, 4 Tsigai stud rams were used and another 4 Tsigai rams were used for teasing. To check the quality of stud rams and for the activation of teaser rams the following procedure was applied. Five culled ewes were treated with intravaginal pessaries of 30 mg fluorogestone acetate (FGA Syncro-Part, 30 mg progesterone analogue, Ceva Animal health, Ltd, France). Sponges were left *in situ* for 14 days and at the time of sponge removal ewes received an i.m. injection of 600 IU pregnant mare serum gonadotropin (PMSG, Ceva Animal health, Ltd, France) to induce oestrus. These ewes were used for semen collection from stud rams and then checking their quality.

Teaser rams were placed with ewes in heat (FGA/PMSG treated) to activate their sexual activity, so that they would seek more actively for ewes in heat at the start of breeding period.

Second group experiment

The experiment was conducted in 2011 and 2012 at the private farm in Momchilovtsi village, Smolyan region. One hundred and nine Karakachan ewes from one of the flocks at the farm aged 1.5 (9 maiden ewes) to 5.5 years were used. As the experiment was carried out at the same farm where the first experiment was performed, all the activities for the separation of the flock into two equal groups (control group, n=55; experimental group, n=54), activation of teaser rams and checking semen quality of stud rams and the application of the salt-free-salt diet were identical (Table 1). The feeding and farming conditions were also identical. Three Karakachan stud rams were used for the insemination, and another 2 rams from the same breed were used as teasers. All rams were 2.5 to 5.5 years-old.

Third group experiment

The experiment was carried out in 2011 and 2012 with two flocks from Bulgarian Dairy Synthetic Population Sheep (crosses of local West Balkan Mountains Sheep x Pleven Blackhead, Awassi and East Friesian Sheep) at the private farm "Elitagro", Ravnishte village, Botevgrad region. Ewes from the two flocks aged 2.5 to 5.5 years, of approximately equal body condition scores (Table 4) were reared in the same grazing conditions. All the ewes were supplemented with 300 g of concentrate mixture (16% CP, no added sodium chloride) per ewe per day. One of the flocks served as control (n =154) and in the other flock (n = 159) the salt-free diet given for 10 days (from 3.08 to 12.08.2011) was alternated with a diet supplemented with 20 g salt per ewe daily for 5 days (from 13.08 to 17.08.2011) . The ewes in oestrus were inseminated artificially, by vaginal cervical administration of fresh semen diluted 1:2 to 1:5, twice in oestrus. The artificial insemination began on the third day after the start of giving 20 g of salt (carefully mixed with the concentrate mixture) per ewe daily for the experimental group. The semen volume and quality of 5 stud rams from the Pleven Blackhead and Awassi breeds, available in the farm, were examined in advance by taking ejaculates twice daily 20 days before the start of breeding period. The quality of sperm (volume, motility and concentration per 1 ml) complied with requirements of the ram's standards. For the detection of ewes in heat, 3 teaser rams for both flocks were used. Teasers were placed in the flock twice daily (in the morning and evening) for 50-60 min each time.

All rams were separated from ewes and reared separately a few months before the beginning of breeding period.

In the beginning of all group experiments, ewes were milked. For the ewes from the first and second group experiment, milking was stopped after the end of breeding period and in the ewes from sheep farm in Ravnishte – at the start of breeding period.

In the three group experiments the ID numbers of ewes in heat and inseminated sheep were recorded on a daily basis for 30 days, as much as continued the artificial insemination (or by the hand service mating system). After that, stud rams were placed in the flocks for natural mating of ewes in oestrus.

Hormone assay experiment: Serum progesterone concentrations in late anestrous ewes, after applying salt-free-salt diet in the experimental group

The experiment was performed at the sheep farm of the Experimental Station of Trakia University, Stara

Zagora from 1.06. to 27.06.2012. Ten ewes aged 3.5 years from the Pleven Blackhead breed with a body condition score of 3.14±0.1 were assigned to two groups (control group, n=5; experimental group, n=5). They were allocated to two indoor pens (each with 5 ewes, equal by age and BCS), where they were fed daily with 1.3 kg alfalfa hay and 0.4 kg concentrate mixture (16,5% CP, no added sodium chloride). According to the literature data (Stankov, 2000), ewes were considered to be in late anoestrus at the time of experiment (June is the end of non-breeding season for Pleven Blackhead breed). The salt-free-salt diet was fed to the experimental group according to the scheme described in the third group experiment (10 days salt-free diet and 5 days 20 g salt per ewe per day). On the 3rd, 10th and 17th day after the start of intake of high doses of salt, in the morning before feeding, blood samples (10 ml) were taken by jugular venipuncture. Blood samples were transferred immediately to the laboratory where they were centrifuged and assayed for serum progesterone. Progesterone concentration (day 3rd, 10th and 17th day after the start of giving high doses of salt) was measured by electrochemiluminescence immunoassay (Elecsys 2010, Roche Diagnostics, Basel, Switzerland) using Progesterone II kits (Roche Diagnostics, Mannheim, Germany) (Bargouli et al., 2007). The detection limit of the assay was 0.03 ng/mL.

Ultrasonographic experiment: Ultrasonographic examinations in the non-breeding and the transitional to breeding periods, after applying salt-free-salt diet to experimental group

First ultrasonographic examination was carried out in late anoestrus with 10 ewes from the Pleven Blackhead breed used in the 4th experiment. According to the scheme described for serum progesterone assays, one of the groups (experimental, n=5) received a salt-free-salt diet and the other served as control (n=5) and received constantly rock salt. Examinations were performed on day 10th (20.06.2012) and 17th (27.06.2012) after the start of inclusion of high doses of salt (20 g per ewe per day) for 5 days (11.06 -15.06.2012).

The second ultrasonographic examination was carried out in 2012, during the transition to breeding season, at the same farm used in the first and second group experiments (12 - 31.07.2012). Forty ewes from the Tsigai breed with a body condition score of 3.45±0.16, divided into two groups of 20 (equal by age and BCS) were used. All the ewes were supplemented with 200 g wheat bran per ewe daily. Grazing and farming conditions of the two groups were also identical. One of the groups served as control and received regularly sodium chloride by providing rock salt ad libitum (n=20), while the other group (n=20) received the salt-free diet given for 10 days (from 12.07 to 21.07.2012) and after that a diet supplemented with 20 g salt per ewe daily for 5 days (22.07 – 26.07.2012).

Ultrasonographic examinations were performed on the 10th day (31.07.2012) after the beginning of high level salt supplementation

Ultrasonographic examinations were performed with the ewe in standing position by real-time B-mode transrectal ultrasonography (SonoScape A6V Co., Ltd, Shanghai, China) equipped with a linear transducer with working frequency from 5.0 MHz to 12.0 MHz. Body condition was estimated at the beginning of all experiments using a scoring system based on a five-point scale from 1 (emaciated) to 5 (obese), as described by Todorov et al. (1994).

The statistical significance of the differences between number of ewes in oestrus and number of corpora lutea at the control and experimental groups was determined by χ^2 (chi square test) by Plohinskiy (1980) and Agresti (2007). The data about progesterone levels were processed by one-way analysis of variance (ANOVA).

Results and Discussion

Because the first and the second group experiments were carried out under similar conditions with two different breeds at the same farm, the results and discussion are presented together.

First and Second Group Experiments

Dynamics of ewes in oestrus and insemination of different groups in the first and second experiment are presented on Figures 1 and 2.

During the first 10 days, in both breeds, a small number of ewes came in oestrus. The expected onset of oestrus, according to literature data (Georgiev, 1996, Stankov, 2000, etc.), in 40-50% of the ewes a few days after giving 15-20 g of salt was not observed.

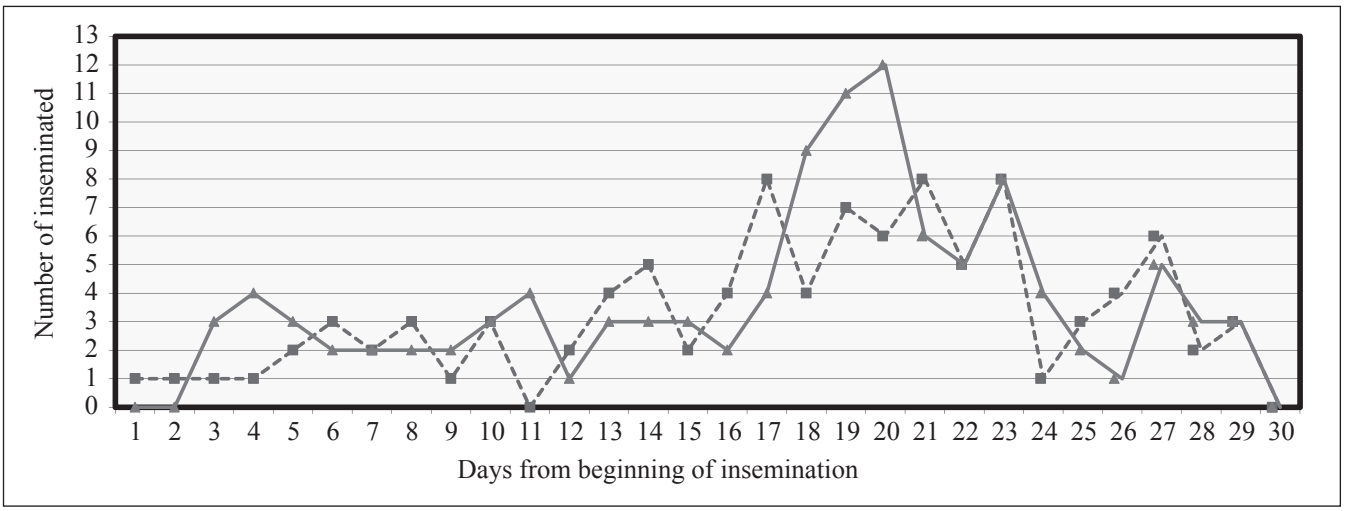

Fig. 1. Distribution of Tsigai ewes in oestrus and inseminated ewes by mating days.
The solid line is for the control group and the dotted line for the group that received the salt-free-salt diet.

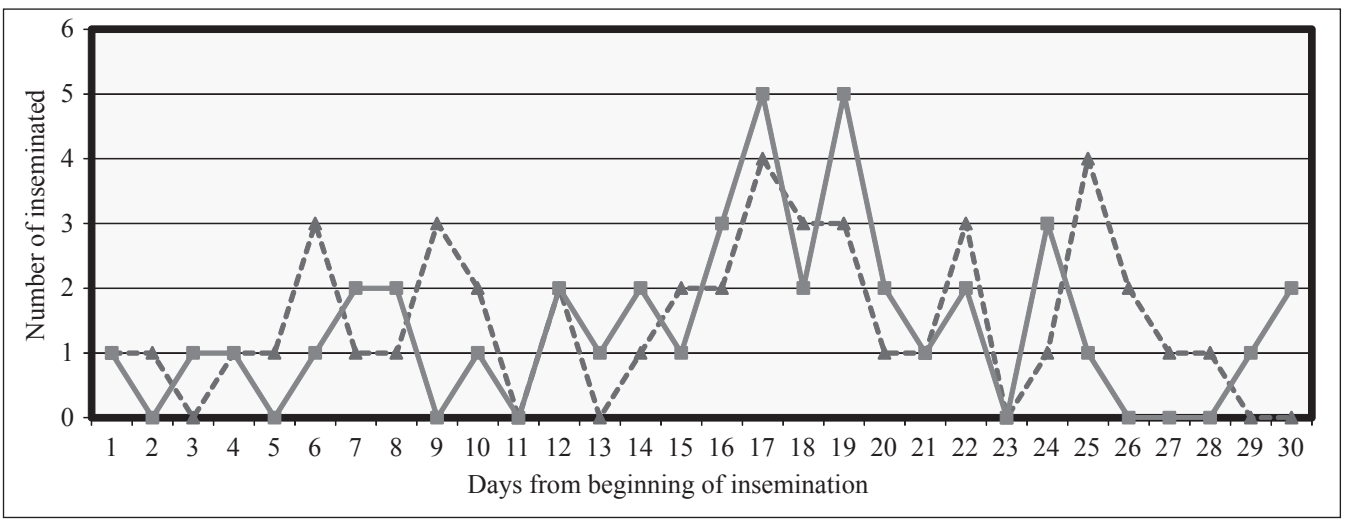

Fig. 2. Distribution of Karakachan ewes in oestrus and inseminated ewes by mating period days.
The solid line is for the control group and the dotted line for the group that received the salt-free-salt diet.

The observed small differences between controls and the group with on salt-free-salt diet were not significant and did not show a definite trend.

Table 1 presents data for the four examined groups of ewes from the first two experiments. During the time of experiments there no ewes were culled because of death or other reasons.

These results are consistent with our previous studies with ewes from Bulgarian Dairy Synthetic Population (Nedelkov and Todorov, 2012). At the same conditions of nutrition and breeding no significant differences in distribution of ewes that manifested oestrus were established between the control groups and the groups of ewes that received the salt-free-salt diet. There were no data indicating the presence of synchronizing effect or stimulation of reproductive axis of ewes in both examined regimens of dietary salt at the beginning of their mating period.

In both breeds included in the first and second group experiments, most of ewes came in oestrus between the

16th and the 27th day after the start of breeding period and placing teaser rams in the flocks. According to literature data and our research this was the period when the "ram effect" occurred (Ungerfeld, 2003; Nedelkov et al. 2011; Todorov et al., 2011).

In our previous studies we observed a better response to the "ram effect" in ewes with BCS over 3.0 (Nedelkov and Todorov, 2012). This gave us a reason to examine the response to the "ram effect" in ewes with different BCS, respectively with BCS <2.9 and >3.0. When considering the response to the "ram effect" according to the different BCS of ewes, determined at the beginning of experiments (at the moment of separation of the flocks into two groups) a tendency for a better reaction of ewes with BCS over 3 was observed (Tables 2 and 3).

Third group experiment

The data from Figure 3 indicate that the first peak of the "ram effect" was lower for the group that received the salt-free-salt diet. Conversely, the second peak was higher at the group receiving high doses of dietary salt compared to the control group. Differences between the

Table 1
Data about groups, ewes in oestrus detected by teasers and the lambing of ewes from different groups♦

Parameters	Tsigai breed		Karakachan Sheep breed	
	Control, number	Salt-free-salt diet, number	Control, number	Salt-free-salt diet, number
Number of ewes in the group	134	133	55	54
BCS#	3.28	3.26	3.16	3.21
Ewes at oestrus (from the 1st to 10th day)*	21	18	9	14
Ewes at oestrus (from the 11th to 15th day)*	14	13	6	5
Ewes at oestrus (from 16th to 27th day)*	69	64	24	25
Aborted, number	2	3	0	1
Barren, number	16	15	6	4
Number of ewes with twins	31	24	20	14
Number of ewes with 3 lambs	0	1	0	0
Live lambs born, number	145	141	68	63
Stillborn lambs, number	2	1	1	0
Number of born alive and dead lambs	147	142	69	63
Number of lambs from 100 lambed ewes (Biological fecundity)	126.7	123.5	140.8	128.6
Number of lambs from 100 available ewes (Industrial fecundity)	108.2	106.0	123.6	116.6

♦ The differences between two groups of the same breed are not statistically significant (P>0.05)

The average body condition score (scale 0 to 5)

* Days after placing teaser rams in the flock and the start of mating period.

Table 2
Ewes responding to the "ram effect" and ewes with manifested oestrus (with BCS <2.9 and >3.0) 16 to 27 days after placing teasers in the Tsigai flock (First group experiment)

BCS	Salt-free-salt diet group			Control group			Total for all ewes		
	Number	Ewes in oestrus, number	Ewes in oestrus, %	Number	Ewes in oestrus, number	Ewes in oestrus, %	Number	Ewes in oestrus, number	Ewes in oestrus, %
< 2.9	44	17	38.6	49	20	40.8	93	37	39.7
>3.0	89	47	52.8	85	49	57.6	174	96	55.2
Total	133	64	48.1	134	69	51.5	267	133	49.8

The differences between two groups are not statistically significant (P>0.05).

The differences between ewes responding to the "ram effect" with BCS <2.9 and >3.0 are not statistically significant (P>0.05).

control and experimental groups as well as between the two peaks were not statistically significant (P>0.05).

Despite the existence of certain differences, which are inevitable in biological experiments, the results did not allow us assuming a significant influence of the salt-free-salt diet on the reproductive cycle in ewes. It is not possible to conclude that the salt-free-salt diet had any significant effect on oestrus synchronization of ewes (Tables 4 and 5).

The differences between groups with BCS over and under 3.0 were not statistically significant at P<0.05. However, for the six groups of the three breeds in group experiments the trend was in the same direction. This allowed us combining data from individual groups of all breeds, which are presented in Table 6.

Although the individual breeds used in the three group experiments had some differences in the average BCS, the results from Table 6 indicate that the improvement of body condition (BCS) of ewes up to 3.0 – 3.5 increased significantly (P<0.05) the response to the "ram effect". On the other hand, in obese ewes with BCS over 3.5, a trend about poor response to the "ram effect" can be discerned.

Hormone assay experiment

Analysis of progesterone in 5 ewes of the control group and 5 ewes that received the salt-free-salt diet were conducted in June, which coincided with the non-breeding season (late anoestrous). As previously discussed, it was reported that a few days after giving high doses of salt most of ewes demonstrated oestrus behavior. Corpus luteum (CL) formation after oestrus and ovulation was followed by increased concentrations of progesterone between days 3 and 7, which then plateau up until ~day 12, and subsequently showed a rapid decline (Bartlewski et al., 1999). Therefore, as a result of the salt-free-salt diet an increase in progesterone levels around 10th day after inclusion of large amount of salt in the diet should be expected.

The results from our experiment showed that one ewe (No. BG 1690377) from the control group had an increased progesterone concentration of 1.796 ng/ml at the 3rd day and 1.529 ng/ml at the 10th day. Such values indicated luteal activity, i.e. a formed corpus luteum. Another ewe from the control group (No. BG 1690422) at the 17th day after the start of inclusion of high doses of salt had an elevated progesterone concentration (1.096 ng/ml), perhaps due to the early luteal phase without reliable signs of manifested oestrus. On the other hand, in the group exposed to the salt-free-salt diet, progesterone concentrations remained very low both on the 10th (0.29±0.03 ng/ml) and 17th (0.38±0.12 ng/ml) day after supplementing 20 g salt to the diet. From the experimental group only one ewe (No. BG 1690428) had an increased progesterone concentration on the 17th day after the start of the high- salt diet, but the value of 0.841 ng/ml was too low to indicate a luteal activity. Despite the variation of progesterone concentration among different sheep breeds (Bartlewski et al., 2011; Chanvallon et al., 2011) the onset of the oestrous season is considered to occur when progesterone concentration is above 1 ng/ml (Zarkawi, 2011; Alnimer et al., 2005). On the other hand, ewes are considered to be anoestrus when the concentration of progesterone is below 1 ng/ml and remain at that level for at least 2 consecutive weeks (Chanvallon, 2011). Our results (Table 7) indicate that the salt-free-salt diet had no effect

Table 3
Ewes responding to the "ram effect" and ewes with manifested oestrus (with BCS <2.9 and >3.0) 16 to 27 days after placing teasers in the Karakachan Sheep flock (Second group experiment)

BCS	Salt-free-salt diet group			Control group			Total for all ewes		
	Number	Ewes in oestrus, number	Ewes in oestrus, %	Number	Ewes in oestrus, number	Ewes in oestrus, %	Number	Ewes in oestrus, number	Ewes in oestrus, %
< 2.9	20	8	40.0	23	7	30.4	43	15	34.9
>3.0	34	17	50.0	32	17	53.1	66	34	51.5
Total	54	25	46.3	55	24	43.6	109	49	44.9

The differences between two groups are not statistically significant (P>0.05).
The differences between ewes responding to the "ram effect" with BCS <2.9 and >3.0 are not statistically significant (P>0.05).

Table 4
Data about main reproductive parameters of the control and experimental groups in the third group experiment♦

Parameters	Control	Salt-free-salt diet
Number of ewes at the formation of flocks (before mating)	154	159
BCS#	2.72	2.70
Ewes in oestrus (from the 1st to 10 day)*	5	6
Ewes in oestrus (from the 11th to 15th day)*	19	11
Ewes in oestrus (from 16th to 27th day)*	71	85
Ewes dead after lambing, number	1	0
Culled and sold ewes without being lambed, number	8	12
Aborted, number	1	2
Barren, number	14	16
Lambed ewes, number	131	129
Live lambs born, number	136	140
Stillborn lambs, number	4	2
Dead before weaning, number	1	2
Number of ewes with twins	9	13
Number of ewes with 3 lambs	0	0
Number of lambs from 100 lambed ewes (Biological fecundity)	106.9	110.1
Number of lambs from 100 available ewes (Industrial fecundity)	88.3	88.1

Average body condition score (scale 0 to 5)
♦The differences between two groups are not statistically significant (P>0.05).

Table 5
Ewes responding to the "ram effect" and ewes with manifested oestrus (with BCS <2.9 and >3.0) 16 to 27 days after placing teasers in the flock of Bulgarian Dairy Synthetic Population Sheep

BCS	Salt-free-salt diet group			Control group			Total for all ewes		
	Number	Ewes in oestrus, number	Ewes in oestrus, %	Number	Ewes in oestrus, number	Ewes in oestrus, %	Number	Ewes in oestrus, number	Ewes in oestrus, %
< 2.9	114	57	50.0	117	50	42.7	231	107	46.3
>3	45	28	62.2	37	21	56.7	82	49	59.7
Total	159	85	53.5	154	71	46.1	313	156	49.8

The differences between two groups are not statistically significant (P>0,05).
The differences between ewes responding to the "ram effect" with BCS <2.9 and >3.0 are not statistically significant (P>0.05).

Table 6
Influence of body condition score on the response of ewes to the "ram effect" (ewes in oestrus 16 to 27 days after placing teasers in the flocks) total for the six groups of the three sheep breeds

BCS	Number of ewes	Number of ewes in oestrus from days 16 to 27	Percent of ewes in oestrus from total number of ewes
under 2.4	61	24	39.3[a]
2.4 – 2.9	302	135	44.7[a]
3.0 – 3.5	198	114	57.6[b]
over 3.5	128	65	50.1[ab]
Total	689	338	49.1[ab]

[ab] The differences between ewes with different BCS are statistically significant at P<0.05 if percent of responding to the "ram effect" is not bearing the same letter

on initiation of oestrus in ewes during the late anoestrous season.

Ultrasonographic experiment

In the control group, examinations conducted at the 10th day of the late anoestrous season (June), the persistence of corpus luteum in one ewe (№ BG 1690377) was found, which coincided with the established high value of progesterone of the same ewe. In the other ewes from the control group there were no changes in the morphofunctional status of the ovaries in examinations on the 10th and 17th day.

In ewes exposed to the salt-free-salt diet at both dates of examinations (10th and 17th day) there were no morphofunctional changes in the ovaries.

In the second experiment carried out during the transition to breeding season (end of July), the transrectal ultrasonography of 20 control ewes established the existence of 11 corpora lutea in 7 ewes. In the group exposed to the salt-free-salt diet the existence of 15 corpora lutea in 9 out of 20 animals was established. The differences between the control group and the group receiving the salt-free-salt diet were not significant statistically (P>0.05). The trend for slightly more ewes with formed corpora lutea from the group that received the salt-free-salt diet was very weak to be of importance.

Ultrasonographic examinations both in late anoestrous and in the beginning of breeding season did not indicate the existence of significant effect of the salt-free-salt diet.

Conclusions

The performed three group experiments with total number of 343 control ewes and 346 experimental ewes of three different breeds (Tsigai, Karakachan sheep, Bulgarian Dairy Synthetic Population) do not give a reason assuming a synchronizing effect of the salt-free-salt diet on oestrus activity of ewes. The differences between control and treatment groups were small and various in individual sheep breeds. Therefore, the salt-free-salt diet, widely recommended in Bulgarian literature is not appropriate for application.

There were no differences of serum progesterone concentrations in the late anoestrus between the control group and the group receiving a salt-free-salt diet, i.e. presence of a synchronizing effect.

Table 7
Serum progesterone concentrations in individual ewes and average value for the group (ng.ml^{-1}± standard error)

Group and number of ewe	BCS	13.06.2012 (3rd day)♦ ng/ml	20.06.2012 (10th day)♦ ng/ml	27.06.2012 (17th day)♦ ng/ml
Control group*				
1. No. BG 1690377	2.9	1.796	1.529	0.185
2. No. BG 1690422	3.2	0.185	0.214	1.096
3. No. BG 0589216	3.0	0.239	0.430	0.229
4. No. BG 1690437	3.5	0.164	0.176	0.157
5. No. BG 0001163	3.2	0.315	0.279	0.427
Mean values	3.16±0.11	0.54±0.31	0.53±0.25	0.42±0.18
Experimental group received salt-free-salt diet*				
6. No. BG 1690399	2.8	0.317	0.355	0.238
7. No. BG 0589204	3.6	0.236	0.267	0.227
8. No. BG 1864470	3.0	0.229	0.217	0.236
9. No. BG 1690428	3.2	0.234	0.355	0.841
10. No. BG 0589214	3.0	0.239	0.273	0.342
Mean values	3.12±0.13	0.25±0.02	0.29±0.03	0.38±0.12*

♦*The days after the start of inclusion of high doses of salt (20 g per ewe per day) for 5 days (11.06 -15.06.2012)*
**The differences between mean values for individual groups are not statistically significant (P>0.05)*

Ulstrasonographic examinations at the non-breeding season and during the transition to normal breeding season did not indicate any significant differences in favour of the salt-free-salt diet as regards to the existence of sexual activity established by the presence of corpus luteum.

There was a better response to the "ram effect" of ewes with BCS 3.0 to 3.5, compare to ewes with lower BCS.

References

Agresti, A., 2007. An Introduction to Categorical Data Analysis. *Jon Wiley and sons Inc.* (Wiley-interscience), Hobouen, New Jersey, USA.

Alnimer, M., M. Tabaa, M. Amasheh and H. Alzyoud, 2005. Hormonal treatments and the ram effect on synchronised oestrus in Awassi ewes at the beginning of the breeding season. *New Zealand Journal of Agriculture Research*, **48**: 473-480.

Bankov, N., L. Kanchev, L. Kostov and K. Vlahov, 1989. Biology and Biotechnology of Reproduction of Domestic Animals. *Publishing house of BAS,* Sofia, pp.73-74 (Bg).

Bargouli, G. G., M. P. Tsantarliotou, C. N. Brozos, N. A. Kokolis and C. M. Boscos, 2007**.** Effect of norgestomet treatment on plasminogen activator activity in the cervical mucus and the endometrium in dairy cows. *Journal of Veterinary Medicine*, **54:** 393-397.

Bartlewski, P. M., A. P. Beard and N. C. Rawlings, 1999. An ultrasonographic study of luteal function in breeds of sheep with different ovulation rates. *Theriogenology*, **52**: 115-130.

Bartlewski, P. M, T. E. Baby and J. L. Giffin, 2011. Reproductive cycles in sheep. *Animal Reproduction Science*, **124**: 259-268

Blache, D., M. J. Grandison, D. G. Masters, R. A. Dynes, M. A. Blackberry and G. B. Martin, 2007. Relationships between metabolic endocrine systems and voluntary feed intake in Merino sheep fed high salt diet. *Australian Journal of Agricultural Research,* **47**: 544-550.

Blache, D., S. K. Maloney and D. K. Revell, 2008. Use and limitations of alternative feed resources to sustain and improve reproductive performance in sheep and goats. *Animal Feed Science and Technology,* **147:** 140-157

Bratanov, K., N. Bankov and T. Iliev (ed), 1975. Intensification of the breeding process in sheep. Zemizdat, Sofia, p.148 (Bg).

Chanvallon, A., L. Sagot, E. Pottier, N. Debus, D. Francois, T. Fassier, R. J. Scaramuzzi and C. Fabre-Nys, 2011. New insights into the influence of breed and time of the year on the response of ewes to the 'ram effect'. *Animal*, **5:10**, pp. 1594-1604.

Georgiev, G. B., 1996. Preparing sheep and rams for the breeding period. In: D. Panayotov (Ed.) "Livestock Handbook, Part II Sheep Breeding ". *Publ. by Trakia University,* Stara Zagora, pp. 95-98 (Bg).

Digby, S.N., D. G. Masters, D. Blache, M. A. Blackberry, P. I. Hynd and D. K. Revell, 2007. Reproductive capacity of Merino ewes fed a high-salt diet. *Animal* **2:9**, pp. 1353-1360.

Doichev, St., T. Kaludina and A. Andreev, 1976. Breeding sheep and hogs in non-breeding season. *Livestock Breeding*, **30** (10): 44-46 (Bg).

Hristova, C. P., 2007. Opportunities for controlling sexual activity of the ewes. PhD Thesis, Agricultural Academy, Research Institute of Mountain Stockbreeding and Agriculture, Troyan (Bg).

Jankov, I., Tz. Hristova and M. Jonikovski, 2004. Results from the application of non-hormonal methods for synchronization of estrus in non-breeding season of milk type ewes. *Journal of Mountain Agriculture on the Balkans*, **7** (4): 350-357 (Bg).

Meintjes, R. A. and R. Olivier, 1992. The effects of salt loading via two different routes on feed intake, body water turnover rate and electrolyte excretion in sheep. *Onderstepoort Journal of Veterinary Research*, **59** (2): 91-6.

Metodiev, N., E. Raicheva and I. Ralchev, 2007. The abilities for combinative treatment of nonhormonal and hormonal methods to achieve synchronized estrus at ewes from Ill de France breed. Proceedings from scientific conference "Tradition and present state of veterinary medicine: University of Forestry, Sofia, pp. 171-176 (Bg).

Metodiev , N., E. Raicheva and I. Ralchev, 2009. Influence of the salt –free –salt diet and the ram effect on main reproductive traits of ewes from Synthetic population bulgarian milk. *Bulgarian Journal of Agricultural Science,* **15** (6): 598-603.

Metodiev, N., N. Todorov and E. Raicheva, 2010. Sexual activity and use of non-hormonal methods for synchronization of fertility and increasing litter size of ewes from Ill de France breed. *Journal of Animal Science,* **47** (3): 15-23 (Bg).

Moinier, B. M. and T. B. Drueke, 2008. Aphrotite, sex and salt – from butterfly to man. *Nephrology Dialysis Transplantation*, **23:** 2154 -2161.

Nedelkov, K., N. Todorov, A. Kolev and T. Marinkov, 2011. Non-hormonal synchronization of estrus in West Balkan Mountain sheep. *Agricultural Science and Technology,* **3** (1): 13 -20.

Nedelkov, K. and N. Todorov, 2012. Influence of the salt-free-salt diet on the synchronization of estrus in ewes from Bulgarian Milk Sheep Population. *Animal Science,* **49** (2):12-22 (Bg).

Nikolov, I., 2008. Biology and Biotechnology of Reproduction of Domestic Animals. *Academic Press of Agrarian University,* Plovdiv, pp. 169-174 (Bg).

Plohinskiy, N. A., 1980. Algorithms Biometrics, *Moscow University Press*, Moscow (Ru).

Slavov, R., 2005. Reproduction of ewes. In: I. Stankov (Ed.) Livestock Breeeding, p. 230, Publ. RIK "Iskra-MI", Stara Zagora (Bg).

Solomonov, H. and H. Jeliazkov, 1976. Attempt for double lambing and tour lambing of young sheep. *Livestock Breeding*, **30** (1): 40-41 (Bg).

Solomonov, H., 1977. Possibilities for increasing of the meat production from sheep. Thesis -Cattle and Sheep Husbandry, Institute of Animal Science-Kostinbrod, p. 206 (Bg).

Stankov, I., 2000. Reproduction of ewes and goats. In: S. Tyankov (Ed.), Sheep and goat breeding, *"Abagar",* Veliko Tarnovo, pp. 376-396 (Bg).

Todorov, N., J. Mitev and R. Otuzbirov, 1994. Body condition scoring in sheep. *Publ. NIS at VIZVM,* Stara Zagora, p. 28 (Bg).

Todorov, N., K. Nedelkov, A. Kolev and T. Marinkov, 2011. Synchronizing fertilization in Bulgarian Milk Sheep Population by the "ram effect". *Animal Science,* **48** (3): 8-15 (Bg).

Ungerfeld, R., 2003. Reproductive responses of anestrous ewes to the introduction of rams. Doctoral thesis, Swedish University of Agricultural Sciences, Uppsala.

Wilson, A. D., 1966. The tolerance of sheep to sodium chloride in food or drinking water. *Australian Journal of Agricultural Research,* **17:** 503-514.

Wilson, A. D. and N. L. Hindley, 1968. Effect of restricted access to water on the intake of salty foods by Merino and Border Leicester sheep. *Australian Journal of Agricultural Research***, 19** (4): 597-604

Zarkawi, M., 2001. Oestrus synchronisation and twinning rate of Syrian Awassi ewes treated with progestagen and PMSG during the breeding season. *New Zealand Journal of Agricultural Research,* **44:** 159-163.

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