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EFFECT OF FOCUSED FLUSHING AT THE END OF THE ANTICIPATED NORMAL LUTEAL PHASE ON SYNCHRONIZATION OF OESTRUS BY INTRODUCTION OF RAM IN THE FLOCK

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Abstract

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The purpose of this study was: 1) to determine the influence of short-time high nutrient intake focused in second part of luteal phase over the response of ewes to the synchronizing oestrus effect of the ram; 2) to determine the effect of a complex of factors known that could influence on the "ram effect". The experiment has been carried out with 344 Pleven Blackhead ewes, divided into two groups. The first group of 168 ewes with average BCS 3.5 and mean live body weight of 66.7 kg, served as a control. The second group of 176 ewes with average BCS 3.2 and mean live body weight of 63.2 kg was put on an intensive short time feeding. Ewes from both groups received 300 g barley per day while the experimental group received an additional 400 g of soybean screenings. In this way, it was investigated whether the lower BCS could be compensated by short-time abundant feeding. The experiment was conducted in the beginning of the natural breeding season of ewes. The artificial insemination started at 06.08.2011. It was observed a delay in the onset of oestrus with two days at the focused (acute) flushed ewes compared with the control group. Perhaps the difference could be associated with the different body condition of groups and with the applied intensive feeding to the experimental group, which may have influenced the lifespan of the last non-ovulatory and ovulatory follicles. Within 11 days, which coincided with the two peaks induced by the "ram effect", ewes in oestrus were 72.0% of all sheep in the control group or 80.1% of all lambed ewes. In addition, at the experimental group the respective indexes are 76.1% and 83.8%. Therefore, with a proper implication of the oestrus synchronization by the "ram effect" it could be achieved 80.1% to 83.8% of inseminated ewes within 11 days. Despite some differences in BCS and live weight between the two groups, it was not found differences in the biological fecundity of ewes.

Key words: focused flushing, oestrus synchronization, luteal phase, ram effect, ewes

Introduction

The introduction of rams just before the end of the anoestrous, after a period of separation from ewes, results in early and relatively synchronous onset of oestrus (Radford and Watson, 1957; Edgar and Bilkey, 1963; Signoret, 1980; Martin and Scaramuzzi, 1983; Knight, 1983; Martin, 1984; Pearce and Oldham, 1984; Signoret et al., 1984; Ungerfeld, 2003).

In previous studies of ours, the shares of sheep in oestrus was reported to be 34.3% (Nedelkov et al., 2012), 45.9% (Todorov et al., 2011) and 49.2% (Nedelkov et al., 2011) within 10-12 days between the 16th and the 28th day of rams introduction into the flocks. In another investigation (Nedelkov and Todorov, 2012) 41.0% of all ewes in the flock have given birth in the "ram effect" period. In experiments of Todorov et al. (2011) and Nedelkov et al. (2011)

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part of the sheep were already in oestrus due to the beginning of the natural breeding period and they were inseminated before expected “ram effect” between 16th and 28th day after introduction of rams. This decreased the share of ewes, which came in oestrus under the “ram effect”. Such sheep would be in heat again when the “ram effect” occurs. This way, the full effect of the applied oestrus synchronisation was not accounted for. Furthermore, in our previous experiments the ewes were relatively underweight (with BCS of 2.5–2.8 in all experiments) which was significantly below the optimal BCS of 3.0 to 3.5 (Todorov et al., 1994; Shindarska, 2011).

It is known that the “ram effect” was dependent on ram libido (Lindsay and Signoret, 1980; Perkins and Fitzgerald, 1994; Rosa et al., 2006). The presence of ewes in oestrus is beneficial for the ram activity (Gonzalez et al., 1991) as well as for the response of ewes (Zarco et al., 1995). With simultaneous introduction of ewes in heat and rams in a flock, a better “ram effect” was observed (Muir et al., 1989).

The effect of high level of nutrient intake by ewes 11-15 days after the introduction of rams, corresponding to the luteal phase of the normal oestrus cycle, after the first or second silent ovulation, is not known, neither how flushing could compensate for the lower BCS of ewes.

In previous studies of ours, the ram libido have not been stimulated, the ewes were thin and those naturally coming in oestrus were inseminated before the anticipated “ram effect” had occurred. This necessitated additional experiments to correct the previous flaws and to establish the potential for oestrus synchronisation through the “ram effect”.

The aim of the present experiment was to test the possibility for oestrus synchronisation in sheep by appropriate application of the “ram effect” and providing the main factors known to be beneficial for this purpose. Second, the study aimed to determine whether the high level of nutrient intake (flushing) for short time, immediately before insemination of sheep with BCS below the optimal ones could improve the “ram effect” and increase the fecundity parameters of ewes.

Material and Methods

Animals. The experiment was conducted in 2011 with 344 Pleven Blackhead ewes aged 2.5 to 8.5 years (inseminated for the second to the seventh time), owned by the Institute of Forage Crops – Pleven. The animals were divided into two flocks – a control group of 168 ewes and experimental group (176 ewes) submitted to high level short-time feeding (from here on called focused flushing). Both groups were allotted by age and fecundity from the previous lambing. During the insemination, all sheep were milked.

Ram libido activation. Seventeen days before rams were introduced in the flock, the oestrus of six culled sheep was synchronised by the following schedule: 14-day treatment with intravaginal sponges containing a progesterone analogue (FGA Syncro-Part, 30 mg fluorogestone, Ceva Animal health, Ltd, France) followed by a single injection of 600 IU PMSG (Ceva Animal health, Ltd, France). After removal of sponges, six teaser rams with aprons (cloth covering the abdomen tied at the withers and the croup) to prevent mating were placed with sheep for 3 days. Due to the hormonal treatment, ewes showed obvious signs of oestrus and were mated repeatedly by rams, which activated their libido. After the preliminary period, activated rams were used for non-hormonal oestrus synchronisation through the so-called “ram effect”. For this purpose, over 15 days, 3 sexually active rams with aprons were introduced in each flock for about 5 hours per day. Except for hormonally stimulated ewes, all other ewes were separated from rams at a distance of 500 m for 2 months. Together with rams, 3 ewes subject of progesterone sponges plus PMSG also were introduced in each of flocks, expecting a second oestrus to occur 1–2 days before the expected “ram effect” and starting artificial insemination of the flock.

Flushing. Apart the daily grazing, all studied sheep were supplemented with a daily amount of 300 g barley two months prior to the artificial insemination. An acute daily feeding with 400 g/ewe soybean screenings (DM 92.66%, CP 38.65%) plus 300 g barley was applied to the experimental group between the 8th and the 15th day after the introduction of teaser rams used to induce oestrus through the “ram effect”. The period of focused flushing corresponded to the end of the anticipated first or to the beginning of the second normal oestrus. The needed amount of feed was prepared on a daily basis by careful mixing of both feeds in a homogenous mix. The concentrate was given twice daily – in the morning at 5.00 AM and in the evening at 5.00 PM during the milking.

Insemination. The artificial insemination was performed by the 16th day after introduction of teaser rams in the flocks. After that stud, rams were placed in the flocks for natural mating of ewes in oestrus. The ID numbers of ewes in heat and inseminated sheep were recorded on a daily basis. The dates of lambing and death cases were also recorded. The five-point scale of Todorov et al. (1994) assessed the body condition score of all sheep subject to artificial insemination. The live body weight at the time of insemination was recorded.

Statistical significance of the differences between control and experimental groups of ewes in oestrus was determined by χ^2 (chi square test) by Plohinskiy (1980). The data about live weight and BCS were processed by one-way analysis of variance (ANOVA).

Results

The artificial insemination continued fortnight from 06.08.2011 to 19.08.2011. During that time, 149 out of 176 (84.6%) of flushed ewes (experimental group) and 127/168 (75.5%) of control ewes were inseminated. The dynamics of insemination showed the two peaks, specific for the "ram effect" (Figure 1).

During the first 11 days of the breeding period including the two peaks, 134/176 focused flushed ewes or 76.1% were in oestrus and inseminated vs 121/168 or 72.0% of control ewes.

A two-day delay in the maximum response of focused flushed ewes to the "ram effect" should be noted.

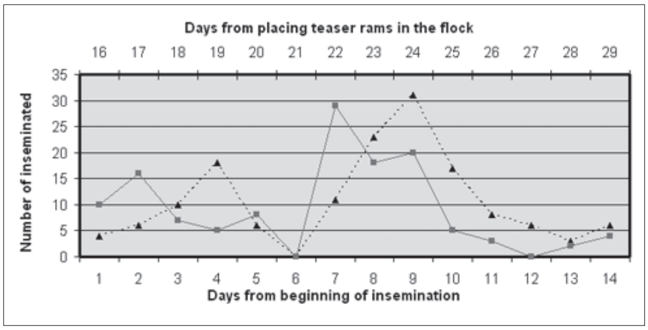

Fig. 1. Distribution of inseminated ewes from control group (solid lines) and focused flushed group (dotted line)

The first peak of insemination was observed between days 16 and 20 after introduction of rams in the flocks. Inseminated sheep were 44 and 46 in the experimental and control groups, respectively. The proportions of inseminated sheep in the first peak from all inseminated sheep within the two "ram effect"-specific peaks (11 days) were 32.8% of focused flushed and 38.0 of control animals (P>0.05).

The second peak of inseminated ewes occurred between days 22 and 26, when 90 of focused flushed or 60.4% of all sheep (artificially inseminated) and 75 of control (59.0%) ewes were inseminated. The differences between groups were not statistically significant (P>0.05).

The effect of BCS on the onset of oestrus during the first and the second peak is presented in Table 1. There was a certain difference between BCS and live weight of animals in the two flocks. In the experimental focused flushed group, the average BCS was 3.2 and live weight – 66.7 kg. In this group, a relatively higher percentage of sheep had BCS <3 (31%) and a lesser percentage were with BCS > 3.5 (22%), compared to controls (17% and 39%, respectively).

In focused flushed sheep, the best response during the first insemination peak was observed in ewes with low BCS (2.2 to 2.9), as 32.7% of all sheep with such BCS came in oestrus within 16–20 days after the ram contact. The trend to onset of oestrus in thinner ewes during the first peak could be related to the focused feeding.

A better response to the "ram effect" was detected in sheep with BCS >3.

Table 1
Effect of body condition score of sheep on their response to the "ram effect" and distribution of ewes responded positively between the two peaks *(BCS, kg ± SEM)

Body condition score		Live weight, kg	n	Inseminated sheep					
				peak I		peak II		peaks I+II	
min-max	mean			n	%	n	%	n	%
Focused flushed									
2.2 – 2.9	2.65±0.02	58.09±0.80	55	18	32.7	22	40.0	40	72.7
3.0 – 3.5	3.15±0.02	62.49±0.66	83	17	20.5	46	55.4	63	75.9
3.6 – 5.0	4.11±0.06	72.54±0.86	38	9	23.7	22	57.9	31	81.6
Controls									
2.2 – 2.9	2.64±0.03	60.06±1.54	28	8	28.6	10	35.7	18	64.3
3.0 – 3.5	3.18±0.02	63.86±0.62	75	19	25.3	36	48.0	55	73.3
3.6 – 5.0	4.16±0.06	72.95±1.07	65	19	29.2	29	44.6	48	73.8
Total									
2.2 – 2.9	2.64±0.01	58.69±0.73	83	26	31.3	32	38.6	58	69.9
3.0 – 3.5	3.16±0.02	63.11±0.46	158	36	22.8	82	51.9	118	74.7
3.6 – 5.0	4.14±0.05	72.79±0.73	103	28	27.2	51	49.5	79	76.7

* the differences between groups were not statistically significant (P> 0.05)

Seventy-four percent of all sheep have responded to the "ram effect", without statistically significant differences between groups. If barren sheep are excluded, which obviously did not came in oestrus due to a variety of reasons or could not be fertilised, 82.0% of the rest of ewes were inseminated within 11 days (Table 2).

The percentage of sheep with twins inseminated during the first 11 days after focus flushing was 44.2% vs. 43.1% in the control group. Having in mind the different BCS between controls (BCS 3.5) and experimental animals (BCS 3.2), the similar percentage of twin lambs indicated a weak positive influence of focused flushing.

The percent of barren sheep was relatively high without statistically significant inter-group difference (Table 3).

In fact, 100 lambed ewes gave birth to an equal number of lambs (biological fecundity). The small difference in industrial fecundity comes from the higher number of dead and aborted ewes in the focused flushed (experimental) group.

Discussion

The dynamics of insemination (Figure 1) showed clearly the synchronizing „ram effect" in both groups of ewes, with 76.1% of focused flushed and 72.0% of control animals being inseminated within both peaks duration (11 days). After 14-day contact with vasectomised rams, 90% of Ile-de France sheep in one experiment were in oestrus during the follow-up 10-day period (Thimonier et al., 2000). The considerably higher percentage was probably related to Ile-de France breed-specific features, as the breed is known to be polycyclic, allowing the fertilisation of part of sheep all the year round. Silva and Ungerfeld (2006) also reported a high share of Corriedale ewes in oestrus (72.9%) between the 17th and the 30th day of insemination.

In our experiment, this high percentage of ewes in oestrus was achieved by using of 3 previously stimulated teaser rams in each of flocks. Lindsay, Wilkins and Oldham (1992) ob-

Table 2
Distribution of sheep that responded to the "ram effect" *

Group (flock)	peak I	peak II	total
Percentage of all sheep in the group (flock) *			
Focused flushed	25.0	51.1	76.1
Control	27.4	44.6	72.0
Total	26.2	47.9	74.1
Percentage from fertilised sheep (lambed + aborted+ dead pregnant) *			
Focused flushed	27.5	56.3	83.8
Control	30.4	49.7	80.1
Total	28.9	53.1	82.0

* the differences between groups were not statistically significant (P> 0.05)

Table 3
Lambing data*

Parameters	Control group*		Focused flushed group	
Number of sheep	168	%	176	%
Mean body condition score	3.5		3.2	
Average live weight kg	66.7		63.2	
Dead pregnant, number	0	0	1	0.6
Aborted sheep, number	1	0.6	3	1.7
Barren sheep, number	17	10.1	16	9.1
Lambed sheep, number	150	89.3	156	88.6
Live lambs born, number	189		190	
Stillborn lambs, number	2		3	
Number of born alive and dead lambs	191		193	
Biological fecundity, %	127.3		123.7	
Industrial fecundity, %	112.5		107.9	

* the differences between groups were not statistically significant (P> 0.05)

served a higher proportion of ewes in oestrus after introduction of 3% or 6% rams as compared to lower number of rams – about 1% of ewe population. In the present trial, teaser rams were approximately 2% of the ewe population. Rodriguez Iglesias et al. (1997) did not succeed to induce oestrus in a higher percentage of ewes by increasing the number of teaser rams from 8% to 16%. Yet, in a more recent study with goats, Bedos et al. (2010) have shown that the response to the male breeder effect depended rather on the aggressiveness and the libido of males than on their number or the duration of contact with females.

The introduction of three already cycling ewes in each flock added to the "ram effect" and resulted in better synchronization. The percentage of ewes exhibiting signs of oestrus and ovulation increased, when sheep in oestrus were introduced in a flock together with rams (Muir et al., 1989).

The two-day delay of the first and second peak of insemination in focused flushed sheep could be hardly explained (Figure 1). Vinoles (2003) suggested that the body condition score could alter the wave-like pattern of ovine sexual cycle as it was observed that all ewes with higher BCS exhibited three waves during the interovulatory interval while in sheep with lower BCS – waves were less numerous. Furthermore, the nutritional treatment of thin ewes prolonged the lifespan of both last non-ovulatory and ovulatory follicles (Vinoles, 2003; Vinoles et al., 2005). In our experiments, thinner sheep in the focused feeding group had probably fewer waves and longer lifespans of last non-ovulatory and ovulatory follicles, which could possibly delay the ovulation onset.

In previous studies of ours, ewes with considerably lower BCS (2.6 in average) showed a lower response to the "ram effect" (Nedelkov et al., 2011; Todorov et al., 2011; Nedelkov et al., 2012). In this trial, the experimental subjects were ewes with optimum BCS for insemination, which improved substantially the "ram effect" response. The good body condition corresponding to BCS between 3 and 3.5 (Bocquier et al., 1988) reduced the intensity of anoestrus and therefore, had an influence on "ram effect" (Thimonier et al., 2000). Despite the lack of experimental data, Folch et al. (2000) affirms that BCS during the insemination was a factor, which could influence the response of ewes to the male breeder. Although focused flushed sheep were with relatively lower BCS than controls, there was no difference in their response, even a slight trend for a better reaction has been detected in intensively fed sheep. In most researches, the aim of flushing was improvement of sheep fertility (Davis, 1981; Teleni et al., 1989; Pearse et al., 1994). Only McCosh et al. (2010) have investigated the effect of the different level of feeding on the response of ewes, synchronized through the "ram effect" method. The authors did not observe any difference between groups, but all sheep were with equal BCS.

Another feature of the study was the magnitude of both peaks. Many authors believe that in general, both peaks should be equal (Martin et al., 1986; Henderson, 1991; Thimonier et al., 2000). Thimonier et al. (2000) demonstrated a lower first peak during the deep anoestrus, probably due to the short luteal phase in a higher percentage of sheep. Khaldi and Lassoued (1991) confirmed the presence of a small first peak in ewes, which were undernourished and thin before the breeding period beginning. Our experiment was conducted in the beginning of the normal breeding season with ewes with optimal body condition, and therefore, none of these factors could have any impact on the distribution of sheep in oestrus in both peaks.

In previous studies of ours, apart the two "ram effect"-specific peaks, small peaks were also observed between the 9th and the 13th day (Todorov et al., 2011). Davila et al. (2011) established that during the normal breeding season, an 11-day contact with rams was necessary to induce oestrus in sheep. Probably, during the longer stimulation period in the present experiment, a higher percentage of ewes reacted to the "ram effect" as early as within days 9-13. After these ewes failed to be fertilised, they exhibited oestrus during the second peak corresponding to the "ram effect", resulting in lower first insemination peak. The character of both peaks could be also influenced by breed-related features (Nugent et al., 1988; Christenson, 1983; Thompson et al., 1990; Chanvallon et al., 2011). It could be hypothesised that more Pleven Blackhead sheep could form incomplete corpora lutea of shorted lifespan and therefore, experienced two silent ovulations reflecting upon the higher second peak.

In both studied groups, almost half of inseminated ewes in the period of both peaks had optimal BCS ranging between 3-3.5. In previous studies, the response of ewes with lower BCS to the "ram effect" was less pronounced (Nedelkov and Todorov, 2012; Nedelkov et al., 2012).

In focused flushed group however, most of inseminated sheep around the time of the first peak were with low BCS. This implies that flushing improved the response to the "ram effect" in undernourished ewes. The intensive nutrient intake for short time was not associated with increase in live weight, but mainly to increase in blood sugar and some metabolic hormones with direct effect on the ovarian function (Scaramuzzi et al., 2006).

In this experiment, the fecundity in the focused flushed group was slightly higher. The effect of flushing on sheep fecundity is mainly anticipated during the first consequent oestrus. As flushing did not result in any significant change in live weight and BCS, it could be barely expected to improve fecundity in sheep fertilized during the second or third oestrus post flushing. Flushing is more important for metabolic hormones (insulin, growth hormone, insulin-like growth factor – IGF-1 and leptin) which are known to influence directly the follicle development and number of ovulated ova, while the effect of the good body condition ("static effect") and its improvement during insemination ("dynamic effect") is mainly mediated via reduction of estradiol level and thus, lower negative effect on FSH secretion. The difference in the pathways of action of the "static" and the "dynamic effects" on one hand, the focused (acute) flushing on the other, necessitates a separate interpretation of fecundity of sheep fertilized during the first oestrus after the focused flushing from the total fecundity of the flock. Sheep fertilised during the first oestrus, i.e. during the first 11 days of the breeding period, which gave birth to twins, were 44.2% in the focused flushed group and 43.1% in controls.

The data gave evidence for an equal fecundity of both groups, despite the difference in the live body weight and BCS, which were higher in control ewes. It is reported that each kilogram increase in live weight resulted in almost 2% improvement of fertility rates (Stankov, 2000; Inskeep, 2002). On this basis, an approximately 6% higher fertility could be anticipated in controls. In fact, the rates were similar, which suggests that focused flushing resulted in a slight improvement of fertility. These results were in discrepancy to data of other researchers, which observed a substantial effect on the number of ovulated ova and fertility in general after focus feeding (Stewart and Oldham, 1986; Teleni et al., 1989; Landau et al., 1995, 1996; Nottle et al., 1990, 1997; Wilkins, 1997; Somchit et al., 2007; Letelier et al., 2008). The differences could be attributed to the good body condition of both experimental and control animals. Probably, the optimal body condition in both groups, achieved through proper long-term nutrition („static effect") is one of the reasons for the mild response to focused flushing. Nottle et al. (1997) also established no effect from focused flushing compared to control sheep (subject to high level of feeding over a long period), but long-term undernourished sheep showed a considerable response. Robinson et al. (2002) assumed that in this experiment, the prolonged feeding of the controls resulted in a „static effect" manifested with attainment of an optimal BCS before the insemination and that this was the reason for the similar fertility rates in flushed undernourished sheep, compared to controls with optimal BCS. Parr et al. (1992) believe that focused flushing could be efficient only in thin sheep with BCS of 2, whereas according to Pearse et al. (1994) and Landau and Molle (1997) focused flushing had an effect on fertility in both sheep in suboptimal and good body condition. According to our experiment, the effect of focused flushing although present is insignificant for sheep with optimal BCS.

When data for the fertility of sheep are interpreted regardless of whether they were inseminated during the first, second or third oestrus, the focused flushing effect fades away. Data presented in Table 3 indicated that biological fecundity was practically the same. This resulted from the weak positive effect of focused flushing in undernourished sheep compared to controls in a better body condition.

Conclusions

The short-time high-level nutrient intake in ewes with lower BCS tended to delay the onset of ram-induced ovulation with 2 days as compared to the control group of ewes, which were with better body condition.

The full application of the "ram effect" including activation of ram libido, placing of several ewes in oestrus in the flock, the lack of fertilisation of ewes in oestrus during the first 15 days of the contact with rams and providing an optimal BCS resulted in best oestrus synchronization results. Having met all these requirements, insemination rates of 74.1% of all sheep or 82.0% of all inseminated sheep for the breeding season were attained within 11 days. These results are by about 50% higher as compared to previous experiments of ours, in which not all of aforementioned conditions were present. Therefore, for a good response to the "ram effect", the following conditions should be present: active rams, good body condition scores of ewes, introduction of several sheep in oestrus in the flock and absence of fertilization of sheep during the first 15 days after teaser rams' introduction.

The effect of focused flushing was more substantial in thinner ewes, while in those with BCS near the optimal (3.2-3.5) the effect was obviously less pronounced.

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