

FLUSHING OR DOE RELOCATION AS BIOSTIMULATION METHODS FOR IMPROVEMENT OF SEXUAL BEHAVIOUR AND PERFORMANCE OF MULTIPAROUS RABBIT DOE AFTER A SUMMER RESTING PERIOD

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ABSTRACT: A study was conducted to clarify the influence of flushing or doe relocation as biostimulation methods on oestrus response and subsequent performance on thirty NZW multiparous 10-11-month-old rabbit does after a two-month resting period in summer. After weaning of their last litter in June, the does were not re-mated and were fed a 100 g daily ration till September, the end of resting period. Then the does were randomly allocated to three groups (10 does/group): a control group (C) in which does remated without oestrus synchronisation, a relocated group (R) and a flushed group (F). Relocation consisted of moving does to another room in cages similar to their initial cages 48 h before the intended mating. F does returned to *ad libitum* feeding one week before mating. After mating, all females were fed and housed under the same conditions. The following parameters were measured: oestrogen level at end of rest period and at mating time, oestrus response parameters, fertility rate, nest quality traits, milk production, litter and kit traits at birth and weaning, and doe weight at mating and kindling. Results obtained revealed that, under the condition of this experiment, flushing was superior to relocation as a biostimulation method, since does of F group responded by significantly higher oestrogen level at mating, improved receptivity and fertility rates, higher milk production, and their litters were heavier at birth and weaning. No significant differences were recorded between the different groups for the nest quality traits. On the other hand, doe relocation did not exert any significant effect on oestrus response and subsequent performance of the rabbit does. In conclusion, a short flushing period of one week could be good biostimulation method for oestrus synchronisation and improvement of receptivity in multiparous restricted fed rabbit does after a long rest period.

Key Words: flushing, doe relocation, oestrus response, nest behaviour, performance.

INTRODUCTION

A limitation to rabbit production is the susceptibility of this species to heat stress, which evokes a series of drastic changes in their biological functions that lead to impairment of production and reproduction during this period (Marai *et al.*, 1999, 2002; Manal, 2005; Ghosh *et al.*, 2008). In Egypt, these effects are accentuated with the high relative humidity during the hottest summer months (Marai *et al.*, 2006). So, as rabbit breeders prefer limiting the breeding season from September to May each year (Marai *et al.*, 1996), rabbit does consequently have a long resting period during which a calculated daily amount of feed (restricted feeding) should be given to keep them in good condition without fattening that would impair their future reproduction (EL-Raffa, 2004).

However, independently of the duration of fasting, caloric shortage negatively affected sexual receptivity and reproductive performance of rabbit does (Brecchia *et al.*, 2006). Interestingly, even mild reduction in the level of nutrition can markedly affect reproduction (Ferguson *et al.*, 2003).

After a resting period, it is advantageous to synchronise their sexual receptivity (Theau-Clément *et al.*, 1998), as reproductive performance of does depends greatly on the percentage of receptive and non-receptive does at the time of insemination (Theau-Clément *et al.*, 1996; Rodriguez de Lara and Fallas, 1999).

Several methods for the synchronisation of oestrus have been proposed to improve receptivity and fertility in female rabbit: these include systematic stimulation of ovarian activity with gonadotrophins (Colin, 1992; Theau-Clement *et al.*, 2008a, b), or administration of PGF2 (Dragan *et al.*, 1996).

Recently, and because of increasing animal welfare concerns, several alternative methods to hormonal treatments, the so-called biostimulation methods, have been developed to synchronise oestrus for a systematic breeding system (Morton *et al.*, 2005; Theau-Clément, 2007).

The sudden transfer of does to another cage shortly before service has been used to stimulate oestrus and so improve reproduction in both nulliparous (Rebollar *et al.*, 1995; Rodriguez de Lara *et al.*, 2000) and lactating (Rodriguez de Lara *et al.*, 2003) rabbit does.

Another means for oestrus induction (biostimulation) can be the change of doe energy status before mating using flushing (Theau-Clément, 2000). Positive effects of restricted and subsequent higher nutrient supply a few days prior to AI on reproduction were recorded in nulliparous rabbits (Eiben *et al.*, 2001; Bonanno *et al.*, 2004; Gómez *et al.*, 2004).

However, the efficacy of these methods has to be confirmed, especially in multiparous rabbit does after a long resting period during summertime. Therefore, this study aimed to evaluate the effect of flushing or doe relocation on oestrus response and subsequent performance of multiparous does after a 2-month summer resting period.

MATERIALS AND METHODS

Animals and location

The experiment was conducted from June to December 2008 in the laboratory animal unit of the Department of Hygiene and Veterinary Management (Cairo University). Thirty multiparous New Zealand White rabbit does (2 previous parturitions from April to June 2008) of about 10-11 months of age and average body weight 2.74 ± 0.21 kg at weaning of their last litter were used.

The does were housed individually in flat deck cages (55×45×30 cm) equipped with automatic drinker, feeding hopper and movable nest box. The ambient temperature and relative humidity ranged from 29 to 36°C and 70-80%, respectively during summer resting period.

Does of all groups were maintained under similar management and hygienic conditions and submitted to 16 h photoperiod per day throughout the experimental period. They were fed a commercial balanced lactating pelleted diet containing 18% crude protein, 13% crude fibre and 2600 kcal of digestible energy per kg (Atmida Feed Company, Egypt).

Experimental procedures

After weaning of their last litter (at June 2008), the does had a 2-month rest period: no mating was done and the does were restricted to 100 g/d feed. At the end of the rest period, does were randomly allocated to three groups (10 does/group) a control group (C) in which does were mated without oestrus

synchronisation, and 2 biostimulated groups: “relocated” (R) and “flushed” (F). Relocation consisted of moving R does to another room in cages similar to their initial cages 48 h before mating then does were taken back to their original cages immediately after service. In the flushed group, F does returned to *ad libitum* feeding one week before mating. From day of mating, both control and biostimulated groups were fed the nutritional requirement of pregnancy and lactation according to the amounts proposed in NRC (1977) for rabbits.

Does of all groups were mated naturally by fertile bucks (male: female ratio, 1-5). When admitted to the bucks' cage, receptive females showed lordosis and allowed mating immediately. Those refused mating were presented to the bucks daily till mating was accepted. Does were palpated for pregnancy diagnosis 10 to 12 d later. Clean nest boxes and nesting materials were provided 4 d before the expected kindling date. Weaning of young rabbits was performed 30 d after kindling. Does were not remated after parturition until weaning of their young. Only one reproductive cycle after resting was analysed.

Hormonal analysis

To study the influence of treatments on mean plasma estradiol-17 β (E2), blood samples were collected in all animals from the ear vein into heparinised tubes, and immediately centrifuged at 1000g, for 10 min, at 4 °C. Plasma was stored at -20 °C until analysed. The blood samples were collected at the end of the resting period for does of the different groups, and again for does of F and R groups only after the end of each treatment (before and after the biostimulation).

E2 concentrations in plasma samples were assayed using a commercial 125I RIA kit (ICN Pharmaceuticals Inc., Diagnostic Division, and Costa Mesa, CA, USA). Plasma E2 concentrations were measured by an immunoenzymatic assay (ELISA) (Silvan *et al.*, 1993).

Reproductive parameters

Oestrus response parameters were recorded as follows. Sexual receptivity was judged at mating time by scoring the colour (white-pink=0, red-violet=1) and turgidity of the vulva (not turgid=0, turgid=1). A doe was scored receptive when its vulva was red-violet (1) and turgid (1), it was considered to be non-receptive in all the other cases (Eiben *et al.*, 2007). Sexual behaviour of does was monitored by recording occurrence of lordosis and number of accepted matings in 10 min from introducing the female to the buck's cage. The acceptance rate (number of does accepted mating/total number of observed does at the first mating trial) was also recorded.

Fertility rate was calculated as the number of pregnant does/total number of does accepted mating \times 100. Kindling rate was calculated as the number of kindled does/total number of does accepted mating \times 100 and prolificacy (total number of live born kits/doe). Nest quality traits were measured on the day of parturition according to Hamilton *et al.* (1997) by observing the nest box and recording the following parameters: nest structure (NS), fur placement (FP), and kit placement (KP) which were scored from 1 to 5 for the first two parameters and from 1 to 4 for the last one; the highest scores of these parameters indicated the best nest quality traits while the lowest one indicated poor nests.

Productive parameters

Doe weight at the end of the resting period, mating and kindling were recorded. Litter and individual kit weight at birth and weaning, weight gain from birth till weaning, litter size at weaning and total preweaning mortality (%) were also monitored. Milk production was estimated by using the regression equation developed by De Blas *et al.* (1995), as follows: milk production (kg)=0.75+1.75 LBW21 (kg); where LBW21 corresponds to live bodyweight of litter at 21 d of lactation which was also recorded.

Statistical analysis

Data were analysed using the ANOVA procedure of the Statistical Analysis System software (SAS, 1996). A T test completely randomised (Student-Newman-Keuls Test) was also used for comparing the difference between means. Results were presented as means±standard error. Non parametric variables (vulva colour and turgidity, receptivity, lordosis, acceptance, fertility, and preweaning deaths) were analysed with a chi-square test (PROC CATMOD of SAS, 1996).

RESULTS AND DISCUSSION*Oestrogen level, receptivity and oestrus response*

Results of this experiment reveal that, when compared with relocated and control groups, a short flushing period at *ad libitum* level has a good biostimulating effect on oestrogen level, receptivity and oestrus response of rabbit does.

As noted in Table 1, at the end of the resting period serum oestrogen level was non significantly different between does of the different groups. However, does of F group showed statistically ($P<0.05$) higher E2 level (60.11 ± 5.41 pg/mL) than either R (35.35 ± 3.28 pg/mL) or C (30.00 ± 3.25 pg/mL) groups at mating time, with no statistical difference between these two latter groups. The increased oestrogen level of F group may be attributed to the increased amount of energy available for the doe which favours the reestablishment of the hypothalamus – pituitary – ovary cycle (Theau Clement *et al.*, 1998; Rodriguez de Lara *et al.*, 2000 and Gómez *et al.*, 2004). However, the shortage of metabolisable fuels and the down regulation of many nutritional mediators due to reduced feed intake, as occurred in R and C groups, may directly influence the steroidogenic capability of ovarian follicles through gonadotrophin- independent mechanism (Brecchia *et al.*, 2006), which resulted in lower oestrogen levels in both control and relocated groups.

It was also noticed that the higher E2 level of F does at mating time was accompanied with significantly ($P<0.05$) improved receptivity and oestrus response. F does responded to flushing process by significantly ($P<0.05$) increased percentage of red violet and turgid vulva, the highest receptivity, lordosis and acceptance rates with increased number of accepted matings compared with either R or C groups (Table 1). Generally, flushing improves follicular growth and oestrus synchronisation (Rodriguez de Lara *et al.*, 2000), while caloric restriction could inhibit through the blockade of the GnRH in FSH and LH secretions (Rodriguez de Lara and Fallas, 1998). Similar findings were obtained by Ubilla and Rebollar (1995) who observed greater concentrations of oestrogen in receptive compared with non-receptive does. Moreover, Rodriguez de Lara and Fallas (1999) found a direct relation between concentration of oestradiol in plasma and vulva colour.

On the other hand, when compared with control does, doe relocation had no significant effect ($P<0.05$) on receptivity indices (Table 1). The lack of effect of relocation may be attributed to the pre-mating caloric restriction, as maintenance of adequate environmental conditions particularly in housing and nutrition may act synergistically with doe cage change to enhance receptivity (Rodriguez de Lara *et al.*, 2003). Brecchia *et al.* (2006) stated that, independently of the duration of fasting, caloric shortage negatively affected sexual receptivity. In accordance with this result, Gómez *et al.* (2004) found no effect for short term cage change on nulliparous NZW rabbit doe receptivity. In contrast, a stimulating effect of short term relocation on receptivity rate of does was recorded in other experiments with nulliparous does which were relocated to another cage 48 h (Castellini, 1996) or only 8 h (Rodriguez de Lara and Fallas, 1998) before insemination.

Table 1: Effect of flushing or doe relocation after summer rest on oestrogen level, oestrus response, fertility and kindling rates, (the number of does on which the parameter was measured appears in brackets).

| | Control (10) | Relocated (10) | Flushed (10) | Significance (<i>P</i> -value) |
|-----------------------------------------------|-------------------------|-------------------------|-------------------------|------------------------------------|
| Ratio of red violet vulva (%) | 50 ^a | 40 ^a | 90 ^b | 0.000 |
| Ratio of turgid vulva (%) | 60 ^a | 60 ^a | 100 ^b | 0.000 |
| Receptivity rate (%) | 50 ^a | 40 ^a | 90 ^b | 0.000 |
| Ratio of lordosis (%) | 40 ^a | 40 ^a | 90 ^b | 0.000 |
| Acceptance rate (%) | 40 ^a | 40 ^a | 90 ^b | 0.000 |
| No. of matings in 10 min. | 1.31±0.56 ^a | 1.50±0.19 ^a | 2.83±0.41 ^b | 0.001 |
| Oestrogen level (pg/mL) at end of rest period | 30.00±3.25 | 28.91±4.25 | 31.60±3.21 | NS |
| Oestrogen level (pg/mL) at mating time | 30.00±3.25 ^a | 35.35±3.28 ^b | 60.11±5.41 ^b | 0.001 |
| Fertility rate (%) | 40 ^a | 40 ^a | 90 ^b | 0.000 |
| Kindling rate (%) | 40 ^a | 40 ^a | 90 ^b | 0.000 |

^{a,b} Means within the same row with different superscripts are significantly different at $P < 0.05$.

NS: non significant at $P < 0.05$.

Reproductive performance

Data of this experiment also showed that the higher oestrogen level and improved receptivity of F does at mating resulted in significantly higher fertility and kindling rates (90%) than either R or C groups (40% for both), but no significant differences were recorded for prolificacy between the different groups (Table 1).

A greater proportion of non receptive as compared with receptive does at insemination had already been shown to reduce fertility and prolificacy in lactating does in the long term (Theau-Clement and Roustan 1992; Theau Clement *et al.*, 1996). Similar results were obtained by Ubilla and Rebollar (1995) and Castellini (1996) who noticed that oestrus behaviour and sexual receptivity at the moment of insemination are related to fertility parameters of the does. Also, Rodriguez de Lara and Fallas (1999) recorded higher kindling rate and total born per litter in receptive than non receptive does. However, a lower nutritional status before insemination reduced the ovulation rate and embryo viability (Theau-Clement, 2000).

The improved reproductive performance of F does is due to the increase in availability of nutrients before oestrous by flushing, as the changes in feed intake and body energy reserves affect the level of those metabolites and hormones which take part in regulation of reproduction (Boiti, 2004; Brecchia *et al.*, 2004, 2006 and Dall'Agilo *et al.*, 2006).

No significant differences ($P < 0.05$) were observed between does of R and C groups for fertility and kindling rates (Table 1) or prolificacy (Table 2). The lower performance of C and R groups may be due to the pre-mating caloric restriction as already stated. Brecchia *et al.* (2006) found that caloric shortage negatively affected fertility rate, but not ovulation rate, litter size and mortality rate at birth. In contrast to this result, Luzi and Crimella (1998) reported an increase in the reproductive performance when pluriparous lactating does were placed in a different cage 48 h before artificial insemination (AI). However, these does were not restricted fed as in our experiment.

Concerning the nest quality traits, our results revealed that the maternal nest traits did not differ between the different groups (Table 2). Manal *et al.* (2004) recorded a significant effect of nutritional treatment on

Table 2: Effect of flushing or doe relocation on prolificacy and nest quality traits, (the number of does on which the parameter was measured appears in brackets).

| | Control (4) | Relocated (4) | Flushed (9) | Significance |
|---------------------|----------------|------------------|----------------|--------------|
| Prolificacy | 6.1±0.37 | 6.00±0.29 | 6.60±0.54 | NS |
| Nest quality traits | | | | |
| Nest structure | 4.00±0.55 | 3.8±0.49 | 4.00±0.52 | NS |
| Fur placement | 3.81±0.46 | 4.03±0.59 | 3.81±0.38 | NS |
| Kit placement | 3.85±0.38 | 3.61±0.32 | 3.76±0.35 | NS |

NS: non significant at $P<0.05$

nest lining that was higher in restricted than *ad libitum* fed rabbits. In rats, Rocha *et al.* (2002) concluded that pup retrieval behaviour of the undernourished mother was not impaired by dietary restriction.

Productive performance

Besides the improved reproductive performance, flushing had a good impact on doe productivity and physical condition. Despite the non significantly different body weight of does of the different groups at the end of the rest period, F group does were the heaviest at mating (2.82 kg) and kindling (2.95 kg) and significantly ($P<0.05$) gave the highest milk yield (3617 g). Kits and litters of these does were the heaviest at birth (59.0 and 385.3 g, respectively) and gained more from birth till weaning (396 and 2278 g,

Table 3: Effect of flushing or doe relocation on productive performance, (the number of does on which the parameter was measured appears in brackets).

| | Control (4) | Relocated (4) | Flushed (9) | Significance <i>P</i> -value |
|----------------------------------------------|------------------------|------------------------|------------------------|---------------------------------|
| Daily feed intake one week before mating (g) | 100 ^a | 100 ^a | 179±20 ^b | 0.000 |
| Doe weight at end of rest (kg) | 2.68±0.49 | 2.66±0.31 | 2.65±0.26 | NS |
| Doe weight at mating (kg) | 2.68±0.06 | 2.67±0.06 | 2.82±0.05 | NS |
| Doe weight at kindling (kg) | 2.79±0.04 ^a | 2.76±0.04 ^a | 2.95±0.05 ^b | 0.02 |
| Milk production (g) | 3199±69 ^a | 3218±57 ^a | 3617±100 ^b | 0.001 |
| Litter size at weaning | 5.00±0.21 | 5.00±0.21 | 5.90±0.40 | NS |
| Litter weight at birth (g) | 318±14 ^a | 314±11 ^a | 385±29 ^b | 0.02 |
| Litter weight at 21 d (g) | 1399±39 ^a | 1410±51 ^a | 1639±73 ^b | 0.003 |
| Litter weight at weaning (g) | 1928±76 ^a | 1941±62 ^a | 2663±183 ^b | 0.000 |
| Litter weight gain at weaning (g) | 1611±65 ^a | 1628±64 ^a | 2278±185 ^b | 0.003 |
| Kit weight at birth (g) | 52.8±1.6 ^a | 52.0±1.0 ^a | 59.0±1.7 ^b | 0.003 |
| Kit weight at weaning (g) | 387±10 ^a | 390±9 ^a | 455±18 ^b | 0.001 |
| Kit weight gain at weaning (g) | 334±13 ^a | 338±9 ^a | 396±13 ^b | 0.002 |
| Prewaning mortality ratio (%) | 18 | 17 | 11 | NS |

^{a,b} Means within the same row with different superscripts are significantly different at $P<0.05$.

NS: non significant at $P<0.05$.

respectively), so they were also the heaviest at weaning (455 and 2663 g, respectively), with the lowest preweaning deaths (11%) compared with R or C groups (Table 3).

The improved physical condition and productive performance of F does could be attributed to the obviously increased feed intake (179 g/d of feed) during the flushing period one week before mating; this leads to heavier body weight with good body energy reserves which resulted in heavier litter at birth and higher milk yield, both in turn reflected in heavier kits and litter at weaning (Eiben *et al.*, 2001) with lower pre-weaning death rates. These results are confirmed by those of Tumova *et al.* (2003), who observed that only in the week immediately after restriction, rabbit show a sharp increase for feed intake, to the extent that the daily gain is higher by 40% than the *ad libitum* fed rabbit.

Finally, it must be emphasised that a limited number of females was used for this study. The response of the flushing was clear but the effect of relocation seems to be inhibited by the preceding restricted feeding. It remains unclear if flushing and relocation have a synergistic effect on oestrus response and sexual receptivity.

CONCLUSION

Although a limited number of females were used, clear responses of flushing on receptivity, fertility and productivity of multiparous NZW rabbit does were obtained on restricted fed does after a rest period (two months or more) during summer months. On the other hand, cage relocation without flushing failed to improve all studied fertility parameters.

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