

Consequences of Calcium Supplements on Rabbit Does Maternal Behaviour and Productive Performance

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Abstract

This study was conducted to assess the influence of three calcium supplements on rabbit does maternal behaviour and productive performance. Twenty adult New Zealand white female rabbits 4-5 months of age were classified into four groups (A, B, C and D) according to the dose of calcium (control, 2.5, 5 and 10 g) added daily to the feeders and each group contains five females. Among four calcium supplementation groups, addition of 10 g calcium carbonate decrease the mean frequency of feeding, the mean does milk production (kg) and means of kits body weight (g) at birth, 21 days and 30 days of age but, increase the means of drinking frequency and duration (min). The higher significant ($P \leq 0.05$) frequency of nursing behaviour was recorded in group B (2.5 g calcium). On the other hand, means of nursing duration (min.), mean body weight of the does (at kindling and at weaning of their litters), the mean litter size (at birth, born alive, still born, at days 21, 30 of age), the total pre-weaning mortality of kits and means of prolactin hormone level (ng/ml) did not significantly ($P \leq 0.05$) different. In light of these results, we could conclude that calcium supplementations have a direct negative effect on ingestive behaviour, nursing behaviour and also, some productive traits of rabbit does.

Key words: calcium, maternal behaviour, rabbit doe, prolactin

Introduction

The life of rabbit does was full of critical periods such as late gestation and lactation (*Manal et al., 2010*) in which a negative energy balance and a large mineral demand could occur which reflected on body condition and reproductive performance (*Fernández-Carmona et al., 2000*). In addition, a mild hypocalcaemia or a syndrome similar to that of milk fever in dairy

cows might occur in highly milk-producing rabbit does (*Barlet, 1980*).

The drain of calcium through the mammary gland creates an imperative need for them (*Gueguen, 1971*). So that, a special attention of feeding requirements such as calcium supplementation was needed of the lactating does to promote the development of the growing rabbits (*Gidenne and*

Fortun-Lamothe, 2002). Although, calcium of the diet did not affect milk production (*Kincaid et al., 1981; Prentice et al., 1995*)

Rabbit does maternal behaviour begins with the construction of a safe breeding burrow as the delivery approach (*Gonzalez-Mariscal, 2001*), lined with both plant materials and her own abdominal fur. She interacts with their offspring by absentee mothering style in which a limited contact by nursing once per day or more than once per day (*Hoy et al., 2004*) for a few minutes unlike most studied mammals (*Numan et al., 2006*).

However, there are very few studies comparing the administration of different dosages of calcium in pregnant and lactating rabbits simultaneously to see any beneficial variation in results helping us to reach to the highest productivity. The aim of this research was to investigate the consequences of calcium supplements on rabbit doe maternal behaviour and productive performance.

Materials and Methods

A total of twenty adult New-Zealand white female rabbits were used in this experiment at 4-5 months of age. Four groups were formed, each consisting of 5 females according to the dose of calcium carbonate added daily to the feeders.

The first group was the control group (group A) where no calcium was added, the second (group B),

third (group C) and fourth (group D) were treated with 2.5, 5 and 10 g calcium carbonate administrated orally per 300 g daily meal (*Patil et al., 2014*). The addition of calcium source started from the day 26 of pregnancy till the 5th day after parturition.

Observations and records

Observation technique:

The behaviour of rabbit does was recorded 24 hrs per day during the whole period of observation (6 days) starting from the postpartum day one to six. For each doe there is a 144 hrs observation period in which we used the focal sample technique (systemic observation techniques in which the observer concentrates its attention on one individual during a certain period of time) as recommended by *Altmann (1974)*.

The following behavioural patterns were recorded:

1. Ingestive behaviour. (*Fraser, 1980*)

a) Eating (consumption frequency and gnawing of pellets duration) (*Prud'hon et al., 1972*).

Consumption frequency means number of times that rabbit enters its head inside the food hopper to take pellets and gnawing means pellets chewing. The mean time (min.) spent in eating pellets per (24 hrs) observation was recorded.

b) Drinking duration and frequency: receiving water from the nipple by frequent movement of the tongue to raise the nipple to allow water flow and this action

accompanied by clicking sound. The mean time (min.) spent in drinking per (24 hrs) observation. The mean duration (min.) and frequency of feeding behaviour and water nipping was estimated in animals from each group for 6 days observation.

2. Nursing display

We focused on nest box visits in the first 2 weeks of lactation of does in the different groups to gain a better understanding of the nursing behaviour of rabbit does in different calcium supplemented groups. Nursing behaviour of rabbit does was observed 24 hrs per day during the whole period of observation. For each doe there is a 144 hrs (6 days) observation period from the beginning of the lighting period on day four of lactation to the end of the dark period on the tenth day (*Matics et al., 2013*). In this way, the beginning and the end of observation coincided among groups. Panasonic WV Ns202ae network video cameras were used for observation. The following data were evaluated:

- a) Number of nursing events per day.
- b) Time of nursing events.
- c) Distribution of nursing events.
- d) Length of nursing events in sec.

A nursing occasion become identified the use of the following standards (*Petersen et al., 1988*):

a- The duration the doe has spent above the nest, the puppies, respectively.

b- The nursing frame position of the doe whilst sitting inside the nest.

c- After nursing, does typically leave the nest speedy, usually with a soar.

Productive and Reproductive performance:(*International Rabbit Reproduction Group. IRRG, 2005*):

1. Body weight of does (kg) at kindling and at weaning of their litters.
2. Litter size: total born, born alive, stillborn, at 21 and 30 days of age.
3. Kits weight (g) at birth, 21 and 30 days of age.
4. Total pre-weaning mortality (%).
5. Milk production (kg) was estimated by using the regression equation developed by (*De Blas et al., 1995*) as follows:

$$\text{Milk production (kg)} = 0.75 + 1.75 \text{ LBW21 (kg)}$$

Where, LBW21 corresponds to live bodyweight of litter at 21 days of lactation which was also recorded.

Serum samples collection (Plate 1).

To have a look at the have an effect on of remedies on suggest serum prolactin level, blood samples were collected from the ear vein of rabbit does into sterilized tubes consistent with the protocol for marginal ear vein blood sample series for rabbits (*Parasuraman et al., 2010*). This technique was usually followed for rabbits in which the animal ought to be placed in a restrainer. Ear was cleaned with ninety five% v/v

alcohol and neighborhood anesthetic cream was carried out on the gathering website 10 min prior to sampling. (If required, the o-Xylene/topical vasodilator may be applied topically on the gathering site to dilate blood vessels). Yellow coloration and baby size canula was used to puncture the marginal ear vein and blood was gathered in a gathering tube at 9:00–10:00 a.m. To avoid circadian variations in feed intake (*Gidenne and Lebas, 2006*). After amassing blood, clean sterile cotton was saved on the collection site and finger stress was carried out for approximately one minute to prevent the bleeding. The gathered blood samples straight away were rcentrifuged at 2000×g for 10 min. Serum saved at -20°C till analyzed. The blood samples have been accrued at day of parturition and at the 21th day postpartum from rabbit does of the specific groups.

Prolactin hormone level estimation.

Prolactin was assayed according to (*Lewis et al., 1992; Gonzalez-Mariscal, 2001*) using a commercial ELISA kit produced by Calbiotech a life science company (PR063f-CBI). The sensitivity of the assay is <0.334 ng/ml.

Data analysis

All data were collected and were evaluated by means of the SPSS 16.0 software package according to (*Argyrous, 2005*) to analyses the differences between groups by using one way analysis of variance F-test (Guthoff et al.), according to (*Cohen, 2002*), means and standard error were used. Duncan's multiple range test was performed to detect significant differences among means when appropriate according to (*Duncan, 1955*). The significance level was set at (P<0.05). A rejection-criterion of 0.05 was set for all statistical tests.



Plate (1): *Blood sample collection in tubes from marginal ear vein of rabbit then centrifugated for obtaining serum samples*

Results

Table (1): Ingestive behaviour frequency and duration (min.) during 24 hrs behavioural observation for 6 days postpartum of rabbit does in different calcium supplementation groups (Means ±S.E)

Behavioural patterns	Group A (control)	Group B (Ca-2.5 g)	Group C (Ca-5 g)	Group D (Ca-10 g)	P-value
Feeding frequency	55.50 ^a ±7.71	54.58 ^a ±7.41	50.42 ^a ±4.00	26.33 ^b ±4.63	0.05*
Feeding duration (min.)	153.25±15.41	153.00±14.76	117.25±15.01	116.33±21.01	0.19 ^{NS}
Drinking frequency	35.22 ^{ab} ±7.90	36.11 ^{ab} ±7.89	27.17 ^b ±4.75	50.22 ^a ±4.67	0.04*
Drinking duration (min.)	30.33 ^b ±4.67	29.67 ^b ±4.51	32.83 ^b ±5.42	54.44 ^a ±6.09	0.004**

Means in the same row with different superscripts are significantly different (P ≤ 0.05). NS: non-significant at P > 0.05 * P ≤ 0.05 ** P ≤ 0.01

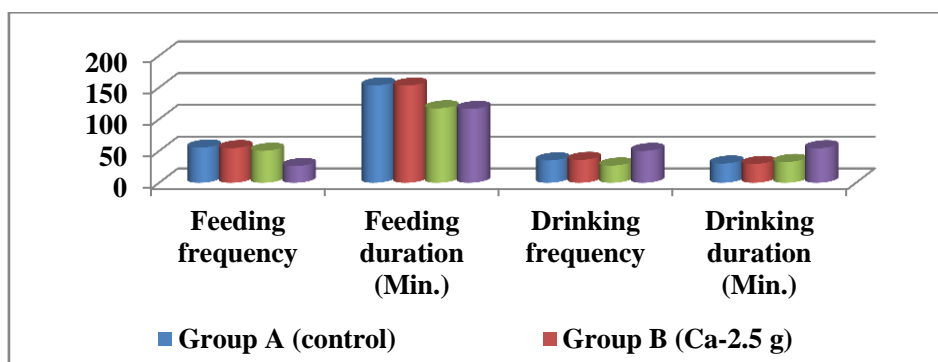


Figure (1): Means of Ingestive behaviour frequency and duration (min.) during 24 hrs behavioural observation for 6 days postpartum of rabbit does in different calcium supplementation groups

Table (2): Nursing behaviour frequency and duration (min.) during 24 hrs behavioural observation for 6 days postpartum of rabbit does in different calcium supplementation groups (Means ±S.E)

Behavioural patterns	Group A (control)	Group B (Ca-2.5 g)	Group C (Ca-5 g)	Group D (Ca-10 g)	P-value
Nursing frequency	1.00 ^b ±0.00	1.38 ^a ±0.20	1.00 ^b ±0.00	1.00 ^b ±0.00	0.02*
Nursing duration (Min.)	2.53±0.13	2.53±0.13	2.48±0.17	2.39±0.16	0.90 ^{NS}

Means in the same row with different superscripts are significantly different (P ≤ 0.05). NS: non-significant at P > 0.05 * P ≤ 0.05 ** P ≤ 0.01

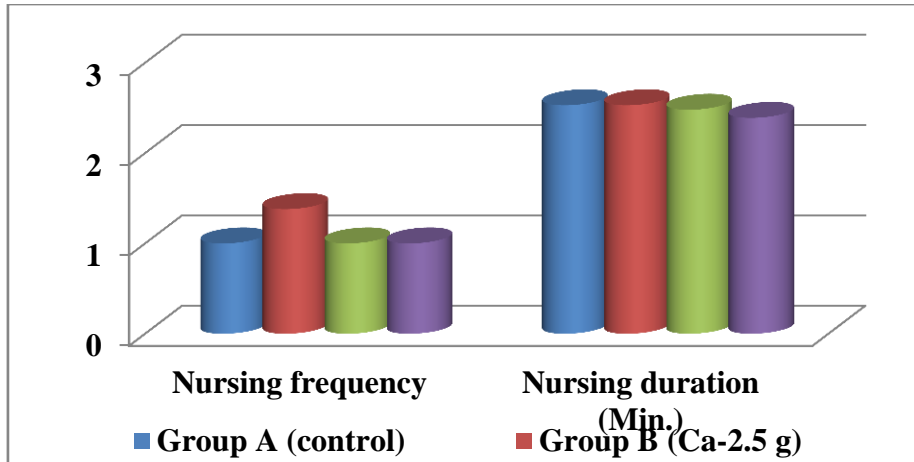


Figure (2): Means of nursing behaviour frequency and duration (min.) during 24 hrs behavioural observation for 6 days postpartum of rabbit does in different calcium supplementation groups.

Table (3): Productive and Reproductive performance of rabbit does in different calcium supplementation groups (Means \pm S.E)

Parameters	Group A (control)	Group B (Ca-2.5 g)	Group C (Ca-5 g)	Group D (Ca-10 g)	P-value
Body weight of does (kg)					
At kindling	3.56 \pm 0.19	3.38 \pm 0.06	3.16 \pm 0.16	3.34 \pm 0.19	0.39 ^{NS}
At weaning of their litters	3.47 \pm 0.20	3.50 \pm 0.10	3.04 \pm 0.23	3.03 \pm 0.28	0.24 ^{NS}
Litter size					
Total born	7.60 \pm 0.68	8.40 \pm 0.51	7.60 \pm 0.24	7.00 \pm 0.84	0.47 ^{NS}
Born alive	7.60 \pm 0.68	8.40 \pm 0.51	7.60 \pm 0.24	7.00 \pm 0.84	0.47 ^{NS}
Stillborn	0.40 \pm 0.24	0.20 \pm 0.20	0.80 \pm 0.37	1.00 \pm 0.32	0.24 ^{NS}
At 21 days of age	6.00 \pm 1.55	4.40 \pm 1.81	7.20 \pm 0.37	5.40 \pm 1.57	0.59 ^{NS}
At 30 days of age	5.00 \pm 1.67	4.40 \pm 1.81	7.20 \pm 0.58	4.80 \pm 1.32	0.52 ^{NS}
Kit body weight (g)					
At birth	63.50 ^a \pm 2.73	51.63 ^b \pm 1.95	57.09 ^b \pm 1.23	55.57 ^b \pm 2.17	0.001 ^{**}
At 21 days of age	312.29 ^a \pm 13.63	293.52 ^{ab} \pm 12.24	278.31 ^{ab} \pm 8.35	270.81 ^b \pm 16.26	0.05 [*]
At 30 days of age	644.60 ^a \pm 28.68	586.50 ^{ab} \pm 33.20	529.20 ^b \pm 16.88	541.96 ^b \pm 37.52	0.02 [*]
Total pre-weaning mortality (%)	4.40 \pm 4.40	6.60 \pm 4.19	8.40 \pm 5.73	8.80 \pm 5.75	0.23 ^{NS}
Milk production(Kg)	5.12 ^a \pm 0.43	4.35 ^b \pm 0.17	4.26 ^b \pm 0.08	3.95 ^b \pm 0.23	0.03 [*]

Means in the same row with different superscripts are significantly different ($P \leq 0.05$). NS: non-significant at $P > 0.05$ * $P \leq 0.05$ ** $P \leq 0.01$

Table (4): Prolactin hormone levels (ng/ml) of rabbit does in different calcium supplementation groups (Means \pm S.E) at parturition and 21th day postpartum

Groups	Prolactin hormone levels (ng/ml)	
	At parturition	At 21 day postpartum
Group A (Control)	1.41 \pm 0.61	44.13 \pm 6.69
Group B (Ca-2.5 g)	1.00 \pm 0.36	60.13 \pm 7.84
Group C (Ca-5 g)	0.69 \pm 0.15	51.38 \pm 8.41
Group D (Ca-10 g)	0.51 \pm 0.02	51.38 \pm 1.90
P-value	0.34 ^{NS}	0.43 ^{NS}

There was no significant difference between means at (P > 0.05)
 NS: non-significant at P > 0.05

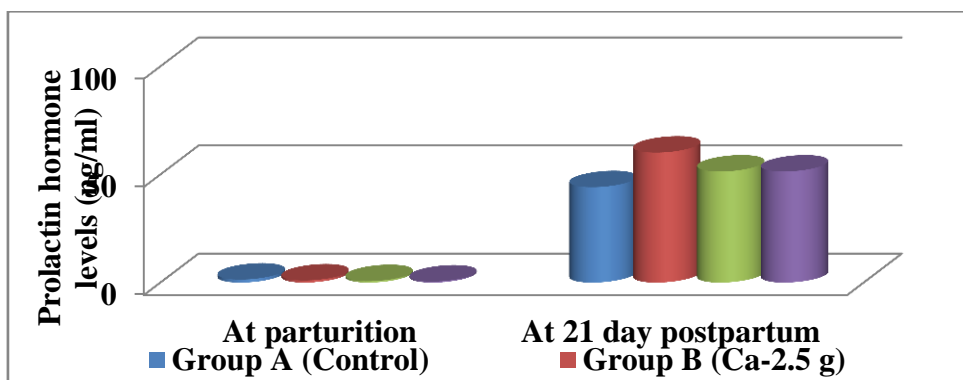


Figure (3): Means of Prolactin hormone levels (ng/ml) of rabbit does in different calcium supplementation groups at parturition and 21th day postpartum

Discussion

Results from table (1) and Figure (1) showed that there was a significant difference (P \leq 0.05) between rabbit does in different calcium supplementation groups (Zero, 2.5 g, 5 g and 10 g Ca/daily meal) in feeding frequency (55.50 \pm 7.71, 54.58 \pm 7.41,

50.42 \pm 4.00 and 26.33 \pm 4.63), drinking frequency (35.22 \pm 7.90, 36.11 \pm 7.89, 27.17 \pm 4.75 and 50.22 \pm 4.67) and drinking duration (min.) (30.33 \pm 4.67, 29.67 \pm 4.51, 32.83 \pm 5.42 and 54.44 \pm 6.09) respectively. Although, there was a slight difference between groups in feeding frequency, the main

negative influence was recorded at the highest calcium concentration. These results in agreement with *Chapin and Smith (1967)* and *Halls (2010)*. There were a higher increase in the frequency and duration (min.) of drinking in the highest calcium supplementation and this may be attributed to higher rates of calcium is excreted through urine according to (*Whiting and Quamme, 1984; Redrobe, 2002; Halls, 2010; Mateos et al., 2010*).

Concerning nursing behaviour parameters as shown in table (2) and figure (2) results revealed that a significant difference ($P \leq 0.05$) between groups in the frequency of nursing. On the other hand, nursing durations (min.) were not affected. This may be attributed to there was no positive effect of added calcium on milk production of does according to *Kincaid et al. (1981)* and *Prentice et al. (1995)*.

The results in table (3) cleared that there was no significant difference ($P > 0.05$) among groups in body weight of the does at kindling and at weaning of their litters. This finding was in agreement with *Shapses et al. (2004)*.

Concerning kits litter size at birth, born alive, still born, at days 21st and 30th of age as showed in table (3) and figure (3) there was no significant difference ($P > 0.05$) between groups. This may be attributed to the total pre-weaning mortality of kits was not different between groups.

Results in table (3) revealed that a significant difference ($P \leq 0.05$) between the control vs. the calcium supplementation groups in kits body weight (g) at birth, 21 & 30 days of age and milk production (Kg.) (63.50 ± 2.73 vs. 51.63 ± 1.95 , 57.09 ± 1.23 and 55.57 ± 2.17), (312.29 ± 13.63 vs. 293.52 ± 12.24 , 278.31 ± 8.35 and 270.81 ± 16.26), ($644.60a \pm 28.68$ vs. $586.50ab \pm 33.20$, $529.20b \pm 16.88$ and $541.96b \pm 37.52$) and ($5.12a \pm 0.43$ vs. $4.35b \pm 0.17$, $4.26b \pm 0.08$ and $3.95b \pm 0.23$) respectively. From the previous findings there was no beneficial effect from adding calcium as the highest results were obtained by the control groups. These results were in agreement with (*Prentice et al., 1995*).

Results in table (4) and figure (3) showed that there was no significant difference ($P > 0.05$) among groups in prolactin hormone levels (ng/ml) at parturition and at 21 day postpartum. This may be attributed to there was no increase in milk production by the added calcium according to (*Kincaid et al., 1981*).

Conclusion

Supplementation of lactating rabbit doe with 10 g calcium carbonate decreases the feeding frequency, does milk production and kits body weight. But, it increases the drinking frequency and duration. Besides, nursing frequency was increased by adding of 2.5 g calcium carbonate. On the other

hand, such conditions do not affect other recorded parameters.

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