

Effect of prenatal overfeeding on post-weaning behavior of rabbits

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Introduction (1)

- ✓ The nutritional environment to which the fetus is exposed during embryonic development could have long-term implications on the function of the appetite-regulating neural network and therefore the way in which an individual regulates energy balance throughout later life **(fetal programming)**
- ✓ Maternal overfeeding during gestation can influence the physiological development of the offspring, although the effects vary considerably, depending on the **intensity** and **duration** of the malnutrition as well as on the **stage of gestation** at which the malnutrition is imposed

Introduction (2)

- ✓ In animal models, exposure to excess nutrient supply during critical windows of fetal development may cause **brain developmental abnormalities** in offspring hypothalamic and hippocampal areas, and in the serotonergic, dopaminergic and opioid systems → increased anxiety, impairment in spatial learning and memory
- ✓ It is therefore assumed that maternal high fat intake could negatively impact aspects of **anxiety related behavior, exploration, learning** and **motivation behavior**

Aim of the study

The effects of does' overnutrition at an early or a late stage of pregnancy on the behavior of kits after weaning

Material and Methods (1)

- ✓ 30 does (Hyla Nouvelle Generation) were artificially inseminated (A.I.) (age of 7.5 month and mean B.W. of 4.52 ± 0.11 kg)
- ✓ 7 days after the A.I., does were randomly allocated to 3 groups:
 1. **C** (Control; **100%** of maintenance needs during the whole period of pregnancy)
 2. **O1** (**150%** of maintenance needs only between the **7th and 19th** day of pregnancy)
 3. **O2** (**150%** of maintenance needs only between the **20th and 27th** day of pregnancy)

Material and Methods (2)

- ✓ After weaning (35th day of age), 48 rabbits were randomly selected (8 per group and sex)
- ✓ Kept indoors in individual cages (0.41 × 0.33 × 0.29 m)
- ✓ Feed and water were provided ad libitum
- ✓ Temperature: 22,5 ± 3,5°C, Relative humidity: 50 ± 20%, Lightning: 12 h/ 12 h light/dark cycle)
- The methods used in the present experiment were approved by the Research Ethics Committee of the Department of Animal Science and Aquaculture of the Agricultural University of Athens under the guidelines of “Council Directive 86/609/EEC regarding the protection of animals used for experimental and other scientific purposes”.

Behavioral Recordings - Cages (1)

- ✓ At the age of 50 and 65 days, behavior elements of 24 rabbits (4 ♂ and 4 ♀ per group) were recorded using 4 video cameras with infrared lighting (TX-14300A, Turbo-X) and data were then stored in a digital video recorder (TX168, Telexper Inc, USA)
- ✓ The behaviors observed could be categorized in the 4 following groups:
 1. **Maintenance** (frequency and duration of **eating** and **drinking**)
 2. **Comfort** (washing, licking, starching)
 3. **Locomotory-investigatory**
 4. **Resting** (sleeping, lying at any position, sitting)



Behavioral Recordings - Open field test (2)

- ✓ At the age of 50 and 65 days old, the 24 rabbits not included in the behavioral recordings in cages (4 ♂ and 4 ♀ per group) were tested in an **open-field test** once a day on 2 consecutive days
- ✓ Open-field test is generally used as a paradigm to measure **emotionality** and as an indicator of **fear**



Behavioral Recordings - Open field test (3)

- ✓ The open field measured **2 x 2 m**, was surrounded by 80-cm high wood walls and was situated in a corner of the barn in which the rabbits were housed
- ✓ Each test lasted **5 min** and after its end, the box, floor and walls were wiped with a cloth moistened with an odorless detergent to remove odor traces
- ✓ For testing, the rabbits were taken out of their pens, placed in a wooden box and carried to the open field. The box was placed in the center of the test area, where the rabbits were released without human contact

Behavioral Recordings - Open field test (4)

- ✓ The behavioral elements recorded during the open-field test were:
 1. **Latency**: Time to first leaving of the central starting area
 2. **Sitting still**: No movement of head, body or legs
 3. **Standing stretched**: The forelegs are forward, the hindlegs stay in place and the heels are visible behind the body
 4. **Moving forelegs**: The forelegs move across the floor whereas the hindlegs stay in place
 5. **Walking**: Forelegs as well as hindlegs move alternately
 6. **Exploration**: Head movements indicating investigation of the environment
 7. **Rearing**: Forefeet are raised from the floor, the rabbit stands on its heels.
 8. **Digging**: Scratching with the forelegs on the floor or wall

Statistical analysis

- ✓ The majority of the studied variables reasonably followed ANOVA assumptions and therefore standard multifactor analysis of variance was employed treating treatment, sex and age of testing and as fixed effects (hour of the day also in cages)
- ✓ In case of variables that clearly deviated from ND, generalized mixed models were employed (zero-inflated negative binomial or logistic regression models)
- ✓ The probability level for mean comparisons was set at 0.05 (Sas/Stat, 2011)

Results (1)

Table 1: Effect of maternal overfeeding on body weights (g) of kits

	Treatment			
Age (day)	C	O1	O2	S.E.M.
0	65.9	70.3	83.1	7.8
14	268.1	266.9	281.2	11.6
35	1024.2	999.6	1014.1	29.0
50	1911.6	1845.0	1914.1	24.6
65	2729.6^a	2614.0^b	2706.6^a	30.6

^{a,b} Means within a row and parameter with different superscripts are significantly different (P<0.05)

Results (2)

Table 2: Effect of maternal overfeeding on feed intake (g/rabbit/day)

	Treatment			
Age (day)	C	O1	O2	S.E.M.
49 th -56 th	161.3 ^a	143.2 ^b	161.9 ^a	4.0
63 rd -70 th	168.1 ^a	150.7 ^b	164.1 ^{ab}	4.6

^{a,b} Means within a row and parameter with different superscripts are significantly different (P<0.05)

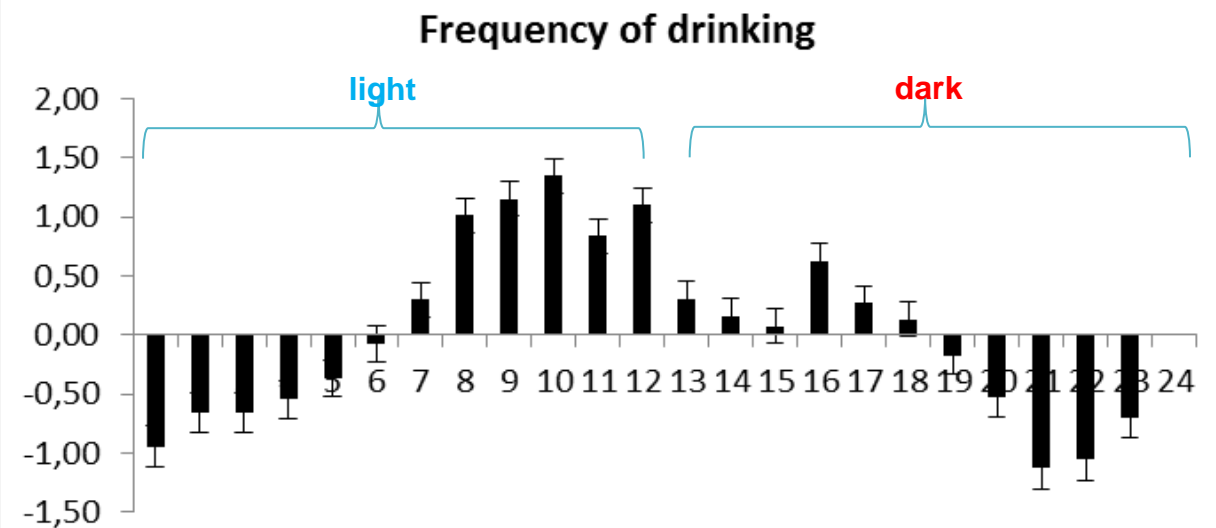
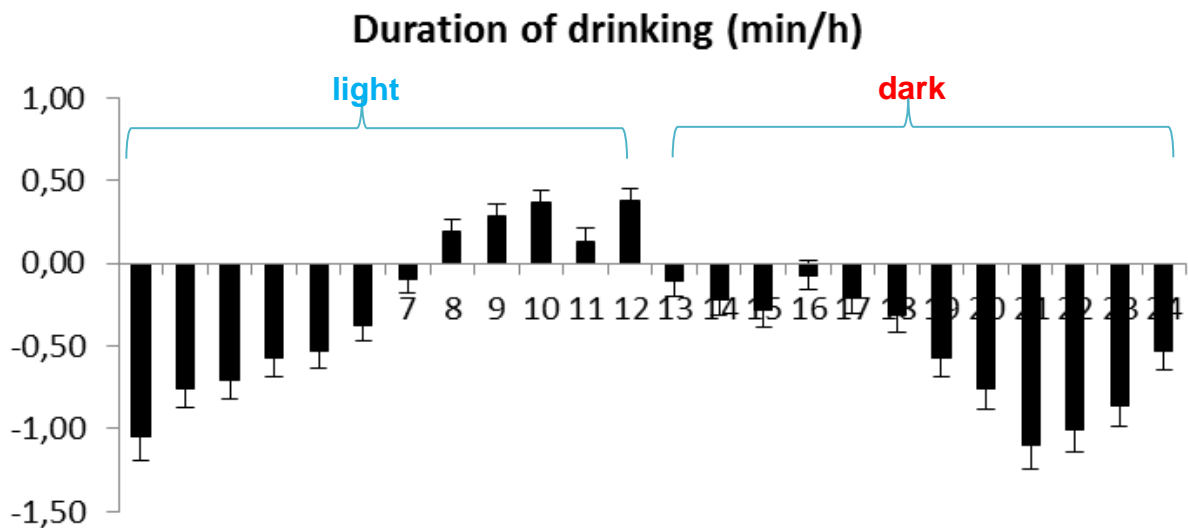
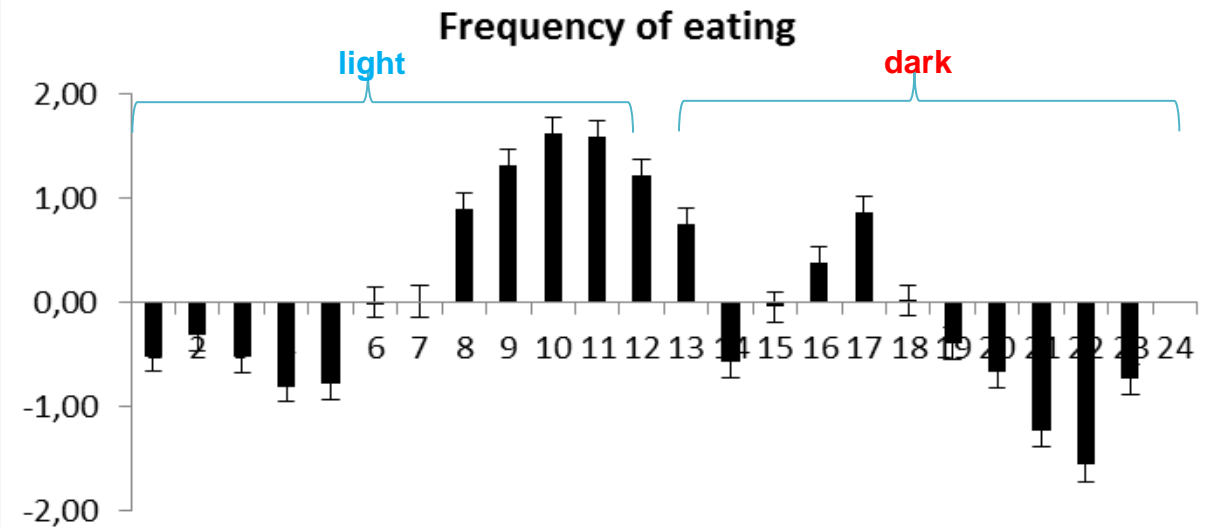
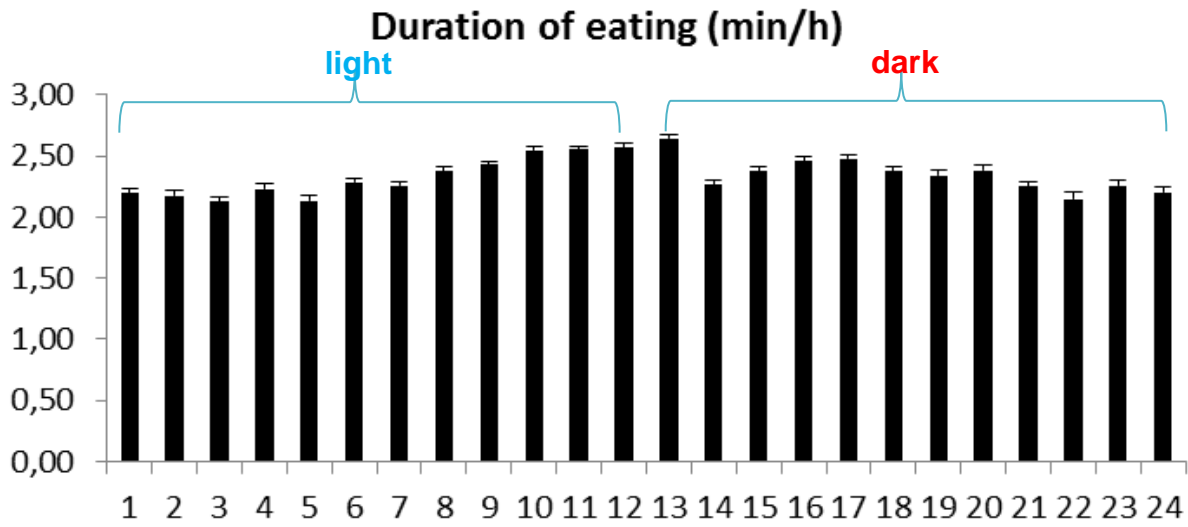
Results (3)

Table 3: Effect of treatment, age of testing and sex on behavioral parameters exhibited in the cages

	Treatment			Age of Testing (days)		Sex	
	C	O1	O2	50	65	Male	Female
Frequency of eating	-0.18 ± 0.04 ^a	0.12 ± 0.04 ^b	0 ^c	-0.49 ± 0.03 ^d	0 ^e	0.08 ± 0.03 ^f	0 ^g
Frequency of drinking	-0.01 ± 0.05	-0.01 ± 0.05	0	-0.18 ± 0.03 ^d	0 ^e	0.04 ± 0.03	0
Duration of eating	2.36 ± 0.01	2.32 ± 0.01	2.32 ± 0.01	2.48 ± 0.01 ^d	2.20 ± 0.01 ^e	2.34 ± 0.01	2.33 ± 0.01
Duration of drinking	-0.28 ± 0.03 ^a	-0.48 ± 0.04 ^b	-0.34 ± 0.04 ^a	-0.30 ± 0.03 ^d	-0.43 ± 0.03 ^e	-0.37 ± 0.03	-0.36 ± 0.03
Comfort behaviours	10.56 ± 0.54	10.97 ± 0.52	10.76 ± 0.56	10.23 ± 0.35 ^d	11.30 ± 0.35 ^e	10.79 ± 0.41	10.74 ± 0.42
Locomotor & Investigatory behaviours	1.96 ± 0.03 ^a	1.75 ± 0.03 ^b	1.83 ± 0.03 ^b	1.88 ± 0.02	1.82 ± 0.03	1.86 ± 0.03	1.83 ± 0.03
Resting	36.27 ± 0.51	37.23 ± 0.52	36.73 ± 0.51	35.02 ± 0.34 ^d	38.47 ± 0.34 ^e	36.73 ± 0.43	36.76 ± 0.43

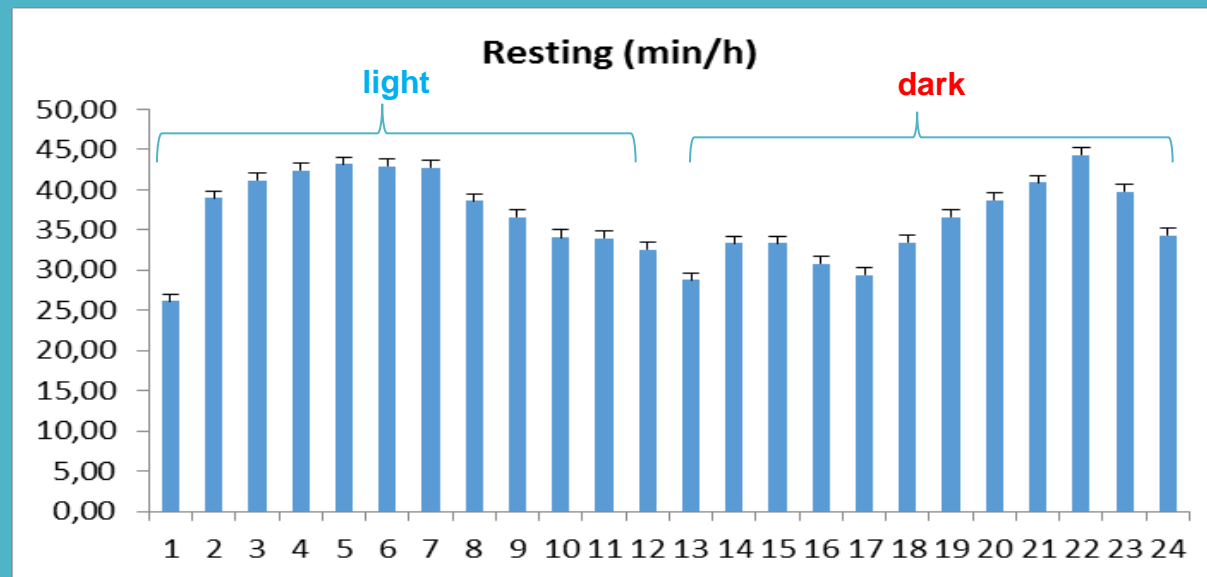
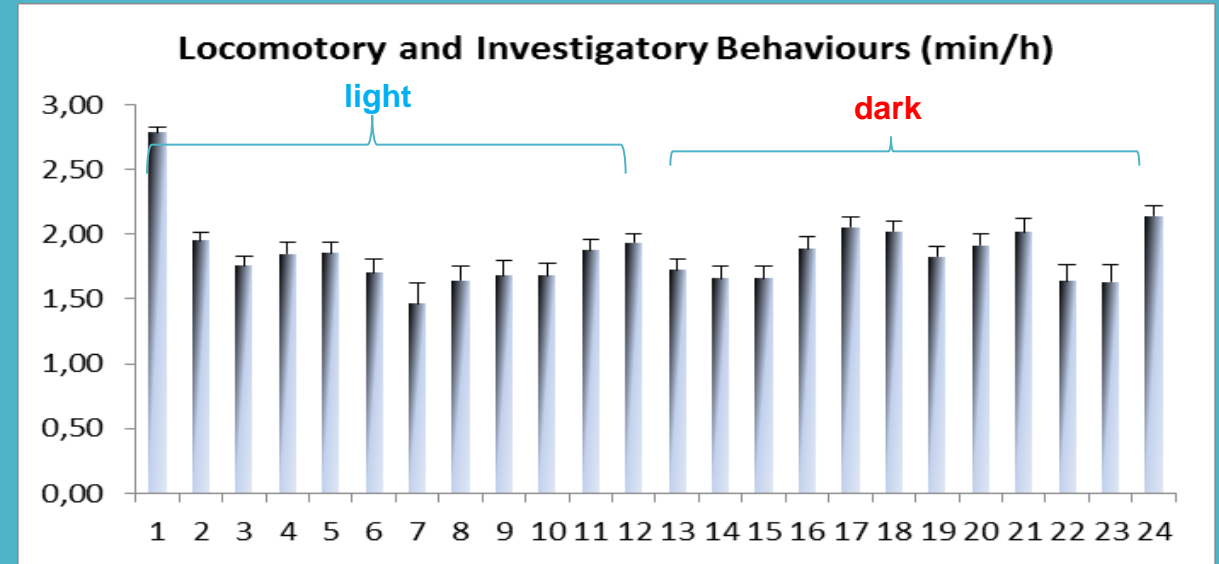
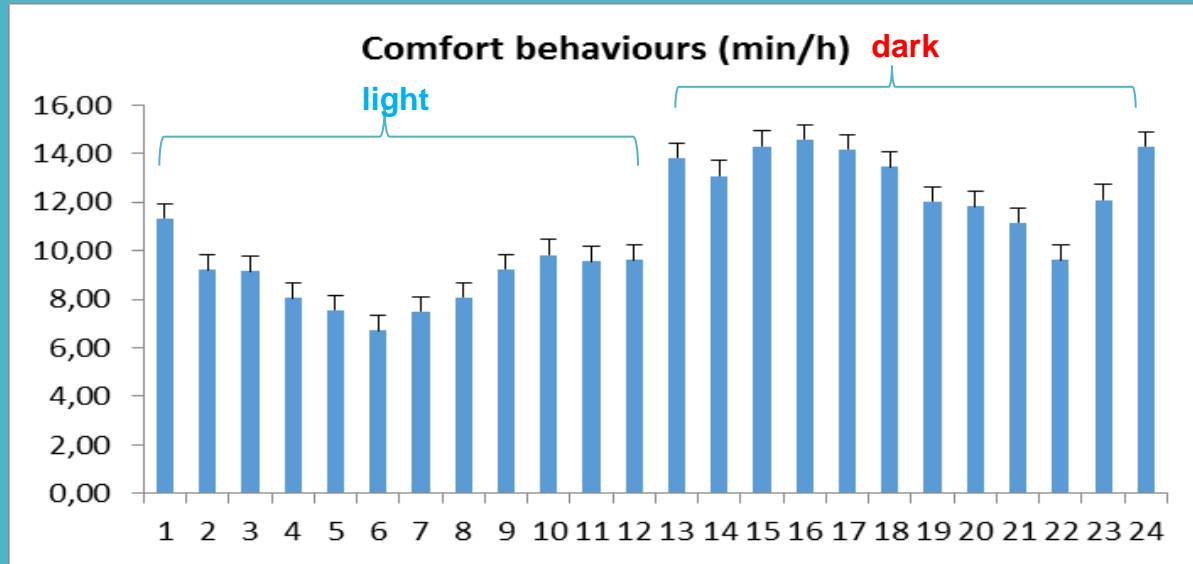
Results (4)

Figure 1: Effect of hour of the day on behavioral parameters exhibited in the cages



Results (5)

Figure 2: Effect of hour of the day on behavioral parameters exhibited in the cages



Results (6)

Table 4: Effect of treatment, age of testing and sex on behavioral parameters exhibited in the open-field test

	Treatment			Age of testing (days)		Sex	
	C	O1	O2	50	65	Male	Female
Latency (log value)	1.62 ± 0.23	1.63 ± 0.20	1.76 ± 0.24	1.66 ± 0.14	1.68 ± 0.14	1.45 ± 0.19	1.89 ± 0.18
Standing stretched	2.72 ± 0.14	2.76 ± 0.11	3.06 ± 0.14	2.96 ± 0.10	2.74 ± 0.11	3.03 ± 0.10 ^c	2.66 ± 0.12 ^d
Moving forelegs	3.11 ± 0.11	3.30 ± 0.09	3.25 ± 0.13	3.44 ± 0.09 ^a	3.00 ± 0.09 ^b	3.33 ± 0.10	3.11 ± 0.09
Walking	124.92 ± 9.80	118.39 ± 8.29	109.63 ± 10.00	141.95 ± 6.12 ^a	93.34 ± 6.30 ^b	125.12 ± 7.84	110.17 ± 7.75
Exploration	135.51 ± 7.65	131.84 ± 6.58	125.25 ± 7.93	155.16 ± 5.43 ^a	106.58 ± 5.67 ^b	136.31 ± 6.22	125.43 ± 6.19
Rearing	2.50 ± 0.14 ^a	3.14 ± 0.11 ^b	3.52 ± 1.13 ^c	3.06 ± 0.09	3.04 ± 0.12	2.99 ± 0.10	3.11 ± 0.10
Digging	3.03 ± 0.04	3.14 ± 0.03	3.15 ± 0.04	3.31 ± 0.06 ^a	2.90 ± 0.07 ^b	3.08 ± 0.03	3.14 ± 0.03
Sitting still	122.85 ± 9.28	113.75 ± 8.01	122.04 ± 9.27	81.56 ± 6.80 ^a	157.54 ± 7.02 ^b	110.65 ± 7.69	128.44 ± 7.81

Discussion (1) - Cages

1. Body weight and feed intake had lower values in O1 compared to C and O2 group of rabbits (at the age of 50 and 65 days)
2. Duration of eating was not different among the experimental groups; however, frequency of eating was higher in O1 compared to the C and O2
3. Locomotory - Investigatory behaviors were exhibited less in O1 and O2 than C group

Discussion (2) - Cages

1. Frequency of eating and drinking had lower values at 50 compared to 65 days; the opposite was found for the duration of these two variables
2. A significant increase of resting time at 65 compared to 50 days was found
3. Frequency of eating was higher in males compared to females

Discussion (3) - Cages

1. Duration and frequency of eating and drinking were greater during the last 4 h of the light period and the first 4 h of the dark period
2. Comfort behaviors were exhibited more during the dark than light period
3. Locomotory - Investigatory behaviors had greater values at the first hour of the light period and the last hour of the dark period
4. Resting values were higher in the light compared to the dark period (nocturnal nature of rabbit)

Discussion (3) - Open-field test

1. Behavioural parameters examined in the open-field test were not significantly different among the treatments (apart from rearing)
2. Rabbits appeared more inactive with age (lower values for moving forelegs, walking, exploration, digging and higher values for sitting still)
3. Males exhibited higher values for "Standing stretched" than females

Conclusion

- ✓ As it is concluded, limited differences (lower values of locomotion-exploration, more meals of shorter duration etc), were found in the exhibition of offsprings' behavior as a result of maternal overnutrition (150%) during pregnancy
- ✓ Further research is necessary for elucidating the processes and mechanisms underlying these effects



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