

Relationship Between Serum Leptin and Reproductive Performance in Rabbits under Different Nutritional Status

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Abstract: The 40 healthy female New Zealand white rabbits aged 30 days were randomly divided into normal control group, fed group and restricted feeding group with 10 rats in each group. Respectively by different feeding methods: the control group was fed with the nutritional standard of rabbits and fed group was increased by 40% on the basis of normal control group while the control group was reduced by 40%. The body weight and serum Leptin level and reproductive performance were measured in each group at the initial stage, the gestation period and the late delivery. The results showed that: in the 3 groups of body weight difference, the greater the weight, the early stage, the early stage of the early emergence of the fed group whose serum Leptin level was 28.23 ng/mL, 8.76 ng/mL (69%), the difference was very significant ($p < 0.01$), compared with 37% 17.65 ng/mL, the difference was also reached a significant level ($p < 0.05$). Feeding group appeared early, sex hormones FSH (Follicle Stimulating Hormone) and LH (Luteinizing Hormone) level is much higher than did not occur in the early stage control group and restricted feeding group and the difference was significant ($p < 0.01$) and limited feeding group and control group was no significant difference between. From the beginning to the late delivery, LH and FSH were first raised and then leveled off and the Leptin was first raised and then decreased and finally, remained relatively stable, apparently with the same changes in FSH and LH. There was no significant difference in the level of FSH and LH between the pregnant women and the late delivery. Pregnancy pregnancy rate; The highest pregnancy rate of feeding group (100%), followed by the control group (90%) and restriction group (50%). Late childbirth of fetal weight: over feeding group and feed restriction group of pups born weight were significantly higher and lower than that in the control group ($p < 0.05$) but nest litter size in between the 3 groups had no significant difference. The and LH levels of the serum leptin levels are associated with increased levels of FSH and Leptin in animals. When the animal is in energy and and has the same change rule. When the animals are in energy and the LH and FSH have the same change rule. When the animals are energetic and and have the same change rule. When the animals are in energy. High levels of the situation, resulting in a decline in the reproductive performance of animals.

Key words: Weight, Leptin, reproductive performance, control group, feeding group, levels

INTRODUCTION

In recent years, the effect of Leptin on the energy metabolism and reproductive performance of animals has become a hot research. Studies have indicated that Leptin plays a role in the adipose tissue and the nervous system not only as a function of the signal but also as a metabolic signal to the reproductive system (Budak *et al.*, 2006). Now the clinical application of Leptin will be very likely to be transferred from the treatment of obesity to the reproductive system of (Airong and Wu, 2007; Weiqiong, 2000) Leptin. The related experiments show that the reproductive function of mice after the treatment with exogenous Leptin, the expression of gonadotropin increased, follicular development, maturation and ovulation, pregnancy as well as the development of testis

and sperm (Muchao, 2000). At present, the mechanism of Leptin regulation of energy metabolism is; Leptin function in the hypothalamus appetite center of the corresponding receptors, causing the secretion of neuropeptide Y decreased, the sympathetic nerve center in the hypothalamus is weakened, resulting in increased excitability and finally led to decreased appetite, food consumption, energy consumption increased. At the same time, Leptin can also act on the leptin receptor in the lower reproductive axis, affecting the synthesis and secretion of GnRH, LH and FSH and by regulating the release of these reproductive hormones to affect the reproductive performance of animals (Qihua *et al.*, 2008). It has been proved that the start of the animal's initial stage and the propagation axis may be regulated by Leptin but its molecular mechanism is not clear. Therefore, it can

provide theoretical basis for the research on the relationship between Leptin and reproductive performance.

MATERIALS AND METHODS

Animal feed: Normal rabbit feed formula; the 32% barley, corn 10, 20% soybean meal, alfalfa hay 25%, hay 10% and additive premix (contain trace mineral elements and multidimensional) 3%. In addition, the addition of 120 g per 50 kg feed. The main nutrients contained in this formula were; crude protein 20%, crude fat 5%, crude fiber 16%.

Experimental animals: The 30 healthy female New Zealand white rabbits were selected and the average weight was (40+0.06) kg, the average weight was (+0.45). The animals were randomly divided into three groups: normal control group (n = 10), fed group (n = 10) and restricted feeding group (n = 10). The condition of feeding: 21-32 in the rabbit room temperature, relative humidity of the rabbit was 30-60% in the whole process of feeding to provide adequate drinking water for each group of rabbits.

Experimental method: By different feeding levels: normal control group was fed with the nutritional standard of rabbits, fed group in the normal control group were fed with the increase of 40% while the control group was reduced by 40%. The body weight and serum levels of Leptin (Leptin), Follicle Stimulating Hormone (FSH) and (LH) were measured in the early stage of pregnancy, pregnancy and delivery. The rabbit after childbirth, reproductive performance records and statistics of each pregnancy rate, litter size, birth weight of newborn rabbits, etc.

Detection index: Clinical observation index; rabbits in each group were observed at the end of feeding, the weight loss, the appetite, the adequacy of the drinking water, the activity of the body, the smooth degree of hair, the smell, color and the color of the urine, the color of urine, the mental state and death. Observe the time and the external performance of each group.

Serum Leptin: In each breeding stage of the experiment, the rabbit serum was kept at 0.5 mL of frozen storage at 70. Leptin was detected by Enzyme linked immunosorbent Assay (EUSA), serum Leptin levels were detected and ELISA Leptin was purchased from the northern Beijing Institute of Biotechnology. Leptin measurement of the concentration of PG/L, the detection sensitivity is 1 L, the

variation coefficient and the coefficient of variation within the group are >8%, each test sample set up the composite hole, operating procedures and steps in strict accordance with the kit and enzyme labeling instructions and precautions.

FSH and LH: In each stage of the experiment, the rabbit serum was kept at 70 for 0.5 mL. The levels of serum Follicle Stimulating Hormone (FSH) and (LH) were detected by the ACS:180PLUS type full automatic chemiluminescence system provided by German Bayer company. The determination of the method of using the method of radioimmunoassay, the use of special reagent boxes in strict accordance with the relevant operating specifications for the determination, the calculation results are processed by natural logarithm conversion.

Reproductive performance: In late pregnancy and childbirth records and statistics of each pregnancy rate, litter size, birth weight of newborn rabbits, etc.

Statistical methods: The experimental data were analyzed by SPSS 17 Statistical Software Package. The final results were expressed as mean+standard deviation(s). The three groups were analyzed by means of the method of multiple variance analysis. The Q test was used to compare the two data.

RESULTS AND DISCUSSION

The detection index of the groups were compared with Table 1-3.

The beginning of each group: From Table 1, it was found that the early stage of the feeding group was 110 days old and the control group and the restricted

Table 1: The contrast of body weight, serum Leptin levels (Leptin) and sex hormone levels in puberty (110 days of age)

	Control group	Excessive feeding group	Restricted feeding group
Oestrus and indexes			
Characteristics of oestrus	No oestrus	Initial estrus	No oestrus
FSH (mLU/mL)	0.075±0.02	0.407±0.15 ^{***}	0.098±0.03
LH (mLU/mL)	0.113±0.03	0.947±0.76 ^{***}	0.086±0.02
Leptin (ng/mL)	8.76±3.67 [#]	28.23±3.01 ^{**#}	17.65±3.87 [*]
Weight (kg)	2.67±0.12 [#]	3.25±0.08 [#]	1.78±0.07 [*]

*p<0.05, **p<0.01, compared to the control group; #p<0.05, ###p<0.01, compared to the restricted feeding group

Table 2: The contrast of body weight, serum Leptin levels (Leptin), pregnancy rate and sex hormone level in gestation

Index	Control group	Excessive feeding group	Restricted feeding group
FSH (mLU/mL)	0.123±0.02	0.160±0.02	0.106±0.01
LH (mLU/mL)	0.228±0.04	0.140±0.03	0.212±0.02
Leptin (ng/ml)	12.968±3.21	20.673±3.14 [*]	18.640±3.71 [*]
Weight (kg)	2.98±0.11	3.56±0.05 [#]	2.25±0.06 [*]
Pregnancy rate (%)	90	100	50

*p<0.05, compared to the control group; #p<0.05, compared to the restricted feeding group

Table 3: The contrast of body weight, serum Leptin levels (Leptin), sex hormone levels and reproductive performance in late delivery

Indexs	Control group	Excessive feeding group	Restricted feeding group
FSH (mLU/mL)	0.083±0.02	0.101±0.01	0.096±0.03
LH (mLU/mL)	0.173±0.03	0.205±0.06	0.195±0.05
Leptin (ng/mL)	10.023±3.61	15.85±3.31*	17.64±3.87*
Weight (kg)	2.76±0.13	3.32±0.18*	2.05±0.11*
Litter size	5.9±0.61	6.1±1.78	5.6±1.86
Birth weight of newborn rabbit (g)	50.69±7.67	58.61±7.23**	43.47±6.75*

*p<0.05, compared to the control group; **p<0.05, compared to the restricted feeding group

feeding group were not in the same time. The initial stage of observation to the normal control group was 152 days and the initial stage of the initial stage of the early stage was 185 days.

Clinical symptoms and reproductive performance: In the process of the experiment, the rabbits in the limited feeding group were much slower, lighter weight, less appetite, thick skin, the body's resistance decreased while the control group and the feeding group increased the weight of the body, the individual is robust (especially, the feeding group). Early stage, control group and oestrus of the feeding group showed obvious: experimental rabbits jumping up and down, bite the cage, vulva is aglow, wetting, swelling and limited feeding group compared with estrous external behavior characteristics is not obvious, only by color and the swelling of the vulva, moist and determine the extent of estrus. Pregnancy groups in estrus has used the same kind of male rabbit breeding, after feeding group 10 female rabbits all pregnancy breeding success, breeding pregnancy rate was the highest, reached 100%, followed by the control group conception rate was 90% and limited feeding group rabbits after repeated mating failed to successfully be pregnant, the pregnancy rate was the lowest, only 50%. Late delivery, group was fed the average litter size limit for 5.6 were lower than the control group just 5.9 and 6.1 feeding group but there were no significant differences between the groups but after feeding group and feed restriction group of pups born weight were higher and lower than that in control group and to differences reached significant level (p<0.05). Compared with the control group and group was fed, after delivery of feed restriction group, the female rabbit generally showed decreased body weight, body significant weight loss, loss of appetite, listlessness, resistance reducing malnutrition.

Body weight: By three groups of rabbits with different feeding methods (normal feeding, excess feeding, limited feeding), the weight of the feeding group was significantly higher than that of the control group (p<0.05) with statistical significance.

Table 4: The changes of FSH, LH, Leptin and body weight in reproduction phases

Index	Puberty	Gestation	Late delivery
FSH(LU/mL)	0.407±0.15**	0.160±0.02	0.101±0.01
LH (mLU/mL)	0.947±0.76***	0.140±0.03	0.205±0.06
Leptin (ng/mL)	28.23±3.01**	20.673±3.14#	15.85±3.31*
Weight (g)	3.25±0.08	3.56±0.05	3.32±0.18

*p<0.05, **p<0.01, compared to gestation; #p<0.05, ##p<0.01, compared to late delivery

Serum Leptin: From Table 1, it can be seen that only in the early stage of feeding group and the serum Leptin level is also higher than the control group (p<0.05) and the serum Leptin level in the restricted feeding group was significantly higher than that in the control group but there was no initial stage. From the data of 2 and 3, the levels of serum Leptin in the early stage of gestation and the limiting feeding group were significantly higher than those in the control group (p<0.05).

FSH and LH: From Table 1, LH and FSH levels were significantly higher than those in the control group () at the beginning of the feeding group and the difference was significant (p<0.01). However, the data of 5-2 and 5-3 showed that there was no significant difference between the FSH and LH levels in each group at the time of pregnancy and delivery.

Changes of FSH, LH, Leptin and body weight in each breeding stage: Taking the feeding group as the research object, combined with Table 1-3 data, the FSH, Leptin, LH and body weight were obtained in the breeding stages (Table 4).

From Table 4, LH and FSH levels were significantly higher than those in the late stage of pregnancy and delivery, especially, in the late stage of labor and the difference of LH was significantly higher than that of ng and Leptin and it was also significantly higher than that of p<0.05 and mL. There was no significant change in body weight at each stage of propagation. From the changes of the indicators, from the beginning to the late delivery, LH and FSH is to rise first then tend to be stable; Leptin is also the first to rise and fall and finally basic remain relatively stable and LH and FSH have the same variation. The change in body weight remained stable with no obvious change.

The relationship between Leptin, body weight and heat cycle: In this experiment, due to feeding group intake more, weight, nutrition and adequate supply of energy is a positive balance of energy and protein, serum Leptin levels were significantly elevated Leptin role at various levels of the lypothalamus pituitary gonad axis, through the promotion of Gonadotropin Releasing Hormone

(GnRH) secretion increased in Luteinizing element (LH), and Follicle Stimulating element (FSH) secretion is increased, so, early start at the beginning of the period, LH and FSH level is significantly higher than that of the control group and feed restriction group. And limited feeding group, although, the serum Leptin level is significantly higher than the control group but due to the intake less, light in weight, nutrition and energy supply is insufficient in negative energy and protein balance, even if serum Leptin levels appear artificially high. On the contrary, Gonadotropin Releasing Hormone (GnRH) secretion was reduced and the inhibition of the Luteinizing Hormone (LH) secretion, so that, early puberty arrival time delay. It can be deduced that when the energy of the animal and the increase of fat reserves, it can stimulate and affect the reproductive process of animals, so as to regulate and control the reproductive activity and reproductive performance of animals.

The relationship between Leptin, body weight, FSH and LH: Studies have indicated that, if the Leptin was added to the culture medium, the secretion of gonadotropin releasing hormone increased but the gene expression of (Morsy *et al.*, 1998) was not changed. But there are also studies showing that Leptin can not directly affect the process of given by Jiyun and Baogang (2001).

Serum Leptin levels were significantly higher than those in control group but FSH and LH were significantly higher than those in control group and LH and FSH were not significantly changed. The Leptin was not the only factor which was also determined by the energy and nutrition supply of animals.

The relationship between Leptin, body weight and reproductive performance: From the trial in the period of late pregnancy and childbirth, after feeding group supply adequate nutrition, weight gain, energy and protein are in balance, serum Leptin increased significantly, promote fetal growth, reproductive performance has been improved, manifested in: the pregnancy rate reached 100%, birth weight of pups was significantly higher than that of control group and restricted feeding group this result is also consistent with relevant reports; restriction restriction group due to malnutrition, weight loss, energy and protein in a negative balance, caused by artificially high serum Leptin and high Leptin levels further exacerbated by malnutrition, reduces animal reproductive performance: conception the rate was only 50%, average birth weight of pups was significantly lower than that of control group and fed group. In conclusion, Leptin can promote the proliferation and the development of the tissues and organs which is the basis for the study of the growth of the fetal cells and the development of the tissues and organs.

CONCLUSION

The emergence of the first stage of the feeling and the nutrition status and serum Leptin level, the more adequate supply, the higher the body weight, the higher the Leptin, the earlier stage of feeling. In the initial stage, the serum Leptin level showed the same trend as the sex hormone FSH and LH, thus suggesting that Leptin may also be one of the inducing factors of animal heat. From the beginning to the late delivery, LH and FSH are first to rise and fall and then tend to be stable, Leptin is also the first to fall and the final basic remain relatively stable and LH and FSH have the same variation. When the animals were in positive balance of energy and protein, more weight, the higher the level of serum Leptin and improve animal reproductive performance (such as conception rate, pups born weight) in a certain extent. On the contrary when the animal nutrition is bad it will be in the negative balance of energy which leads to the decrease of body weight and the deficiency of serum Leptin level.

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