

Effect of sedative drugs with vitamin B₆ combined with other B vitamins in the anti stress of captive amur tiger

Qiao Zhenglei^{1,2} and Ma Jianzhang¹

¹College of Wildlife Resources, Northeast Forestry University, Harbin, China

²College of Life Science and Technology, Mudanjiang Normal College, Mudanjiang, China

Abstract: In order to reduce the stress response of animals in animal transportation, it is often used to feed the animals with sedative drugs combined with compound vitamin B. In this article, we will randomly divide 16 amur tiger into the control group and the experimental group. The drug intervention is carried out in the experimental group. The sedative is mainly dexmedetomidine, combined with compound vitamin B, while control group did not feed the drug. The urine of two groups of individuals was collected before and after transportation and the cortisol content in urine was determined by radioimmunoassay as an indication of stress. Finally, referring to the changes of cortisol level in amur tiger, we analyzed the effect of vitamin B₆ combined with sedative drugs on the anti stress effect of amur tiger. The results showed that the cortisol level in the control group was significantly different ($P < 0.05$), but there was no significant difference in the cortisol level in the experimental group ($P > 0.05$). Conclusion: this kind of veterinary medicine which is mainly composed of sedative dexmedetomidine and vitamin B₆ is obviously helpful for reducing the stress of the Amur tiger in transferred transportation.

Keywords: Sedative drugs, amur tiger, vitamin B₆, cortisol, stress.

INTRODUCTION

Monitoring of the physiological state of animals is beneficial to the timely grasp of the physical condition of animals and the discovery of unfavorable factors in order to better manage the animals (Ganswindt *et al.*, 2003; Good *et al.*, 2003). At the same time, it can make the animal in a better state to the greatest extent and improve the welfare of the animal. Steroid hormones in urine and feces have long been used to monitor the reproductive cycle of animals, and there are many studies on glucocorticoids (Goymann *et al.*, 1999; Goymann *et al.*, 2002). Cortisol, is recognized as indicators of animal stress; physiological studies showed that the physiological stress and cortisol in the body of the animal can indicate, the level represents the degree of stress of animal body (Hunt *et al.*, 2004; Hierbert *et al.*, 2000); at the same time, affected by external environmental factors affecting cortisol is relatively large, such as high temperature and transportation stimulating factor can lead to animal blood cortisol (McDonald, 1980; Millspaugh *et al.*, 1996). The monitoring of cortisol level is beneficial to the analysis of the source of stress and the degree of stress in the animal body, so as to better protect the animals and release the "pressure" of the animals.

Animal stress reaction caused by larger in transfer and transport, such as often, roaring restlessness and even convulsions, sedative to reduce animal stress will also bring greater side effects (Wasser *et al.*, 2000; Plame *et al.*, 2000). At present, domestic animal feed in animal transfer

and transportation in order to reduce the stress reaction of the animal, often used mainly containing sedative drugs also contain vitamin B₆ to feed the animal, no side effects, but also necessary for a healthy organism (Wasser *et al.*, 1997). It has been confirmed that glutamate in the brain increased and convulsion, and vitamin B₆ is glutamic acid decarboxylase coenzyme, glutamic acid can catalyze the formation of GABA in the brain, it belongs to an inhibitory neurotransmitter, has the function of inhibiting excitatory receptors and blocking ganglion synaptic transmission, thus the antispasmodic and promoting effect of static, improve the anti stress ability. Vitamin B₆ has been reported in the human body for sedative, anticonvulsant, and even treatment of epilepsy, but there is no report on anti stress in animals (Wingfield *et al.*, 2001). Previous studies have shown that vitamin B₆ has a significant effect on the treatment of convulsive diseases, and some even effective rate is 92% (Good *et al.*, 2003). The static drops of vitamin B₆ 100mg/ (kg d) stop no longer relapse. The effect of intravenous drip of large dose of vitamin B₆ is better than common antistartled drugs, no side effects, no influence on respiration and circulation, and it is suitable for patients with frequent convulsions and routine anticonvulsant drugs (Goymann *et al.*, 1999). At the same time, vitamin B₆ can be used as an adjunctive drug to combine Levetiracetam to treat epilepsy and improve Levetiracetam induced behavioral abnormality (Zhang, 2015). Amur tiger (*Panthera Tigris altaica*) is a class I key protected animal in China. It is listed as endangered class (EN) by the international natural and Natural Resources Protection Union (IUCN). It is listed in Appendix 1 by the international trade organization of endangered species (CITES). The stress of

*Corresponding author: e-mail: jianzhangma@163.com

the process of captive Amur tiger was transferred during transportation and understanding, help provide better transport and transport as early after the condition, reduce the stress response of the tiger, also is helpful to the correct analysis of the adverse conditions after the emergence of tiger transport.

MATERIALS AND METHODS

Research objects

From January 2014 to February -2017, 16 female adults (8 females, 8 males) were transported and transported to adult females and Amur tigers. The center is located at 129°02' 08" E, 44°48' 47" N, Belong to the cold and humid climate in the high mountains, frost free period of 90-115 days, the average annual sunshine hours 2339.8h, the annual average temperature of 6.1°C, the average rainfall of about 670mm. The breeding center covers an area of 250000m², a total of more than 300 adult Amur tiger cubs 200. During the day, the 9:00-9:30 tiger was put to the outside, and the 15:30-16:00 received the food and water in the house. Compound vitamin B can be dissolved into the water of the tiger, and the time is continuously fed for 5 days when the tiger is recovered 1 days before transportation. The main component of compound vitamin B soluble powder for vitamin B6, also contains a small amount of B1, B2 and nicotinamide (called vitamin B3) and calcium pantothenate (vitamin B5, purchased from the nickname) Jiangxi ambitious Animal Pharmaceutical Co. Ltd., the registered number of approval for veterinary word (2012) 140026071, the dissolution rate is 3g compound vitamin B soluble powder dissolved in 10kg water. In this study, 8 male tigers named respectively by Park No. 156, No. 271, No. 305, No. 589, No. 599, No. 695, No. 709 and No. 993, 8 females were named by Tiger Park No. 149, No. 195, No. 260, No. 342, No. 446, No. 588 No. 1006, No. 1164; all the call by the name tiger breeding center. The animal experiment program has been approved by the experimental animal ethics committee, which conforms to the principles of animal protection, animal welfare and ethics and conforms to the relevant provisions of the national laboratory animal welfare ethics, No.WSKZPF/12ED.

Composite vitamin B soluble powder

The name of the drug used in this article is *composite vitamin B soluble powder*; the manufacturer is Jiangxi Hong Tong Animal Pharmaceutical Co., Ltd. The approval number is veterinary drug (2012) 140026071 and the implementation standard is 1435th of the ministry of agriculture announcement, the quality standard of compound vitamin B soluble powder. In composition, every 1g vitamin B containing vitamin B1 is 3.0mg, B2 is 0.6mg, nicotinamide (other name vitamin B3) is 3.0mg, calcium pantothenate (other known as vitamin B5) is 0.3mg, B6 for 0.9mg, and the rest is glucose. The use of the method is to add 3g vitamin B soluble powder in each

10kg water and the time is continuously fed for 5 days before transportation.

The method of collecting and preserving urine

Fresh urine was collected by 10ml syringe and stored in a number of 2ml centrifuge tubes for storage at 20°C. Because the tiger urinating every day and having a large amount of urine, it is very convenient to sample. In order to reduce the surface pollution, absorb the intermediate urine, such as a small amount of urine, and then absorb the surface urine. After transportation, the sampling time of urine samples began for 5 days from the day of transportation, and the time of urine sampling before transportation was 5 days before transportation. Each individual was collected 1 times a day without a period of time, taking first times of urination in the morning.

Determination of hormone

After the antibody was added to the standard samples and the samples, incubated at 37°C for 1hr, 15min and 3800rpm were added to 15min, The corticosteroid radioimmunoassay kit produced by the northern Biotechnology Institute of Beijing was used to determine the content of cortisol in the urine. Two tube parallel samples were measured for each sample. The XH6080 type radioimmunogamma recorder produced by the Xi'an nuclear instrument factory was counted. The main technical parameters of the kit are: the detection range is 0.1-500ng/mL, the sensitivity is 1.0ng/mL, the coefficient of variation is in batches <5% (n=10), between batches is < 10% (n=10), and the average recovery rate is 98%.

Data analysis method

The results of cortisol determination of male and female tiger were divided into two groups: the control group and the experimental group. The independent samples T test (normal distribution) and two independent samples test (non normal distribution) in the nonparametric test were carried out by SPSS17.0 software. If there is no special annotation, it is a double tail test and a significant level of $\alpha = 0.05$. The data is recorded as an average value of standard error (Mean \pm SE), and all values are reserved for a decimal number.

RESULTS

Cortisol levels and transport compared to the control group and the experimental group male tiger transport in human urine after the former majority have varying degrees of increase, the control group in exon 156 of other male tiger after transport than before the transportation of cortisol levels increased significantly ($P < 0.05$), in addition to the experimental group 993, cortisol levels compared with before transportation slightly after transport decline, other male tiger cortisol levels increased were not significant ($P > 0.05$) (table 1). That all male tigers have different degrees of stress reaction in the

Table 1: Cortisol level comparison of male tigers before and after experiment (ng/mL)

Group	No.	Before experiment	After experiment	P
Control group	No. 156	69.1±6.9	80.4±7.0	P>0.05
	No. 305	75.5±9.4	115.3±9.6	P<0.05
	No. 589	92.8±5.6	125.1±6.9	P<0.01
	No. 709	72.4±4.1	102.4±8.2	P<0.05
Experimental group	No. 271	109.7±5.1	124.0±6.3	P>0.05
	No. 599	82.4±7.3	94.0±8.3	P>0.05
	No. 695	66.9±5.8	81.0±7.2	P>0.05
	No. 993	112.9±9.5	107.9±8.2	P>0.05

Table 2: Cortisol level comparison of female tigers before and after the experiment (ng/mL)

Group	No.	Before experiment	After experiment	P
Control group	No. 149	22.8±2.8	41.5±6.3	P<0.05
	No. 195	61.4±8.4	98.3±9.3	P<0.05
	No. 342	67.4±8.5	110.9±9.2	P<0.01
	No. 1006	89.7±6.0	121.8±7.8	P<0.01
Experimental group	No. 260	113.5±6.5	131.2±10.7	P>0.05
	No. 446	62.0±9.7	71.1±9.1	P>0.05
	No. 588	60.3±11.5	79.3±11.0	P>0.05
	No. 1164	135.6±10.1	156.9±12.9	P>0.05

transport, but the experimental group was significantly less than the control group, in order to prove this sedative compatibility of vitamin B6 based compound vitamin B has obvious effect on the reduction in the transfer and transport stress in male tiger. No matter in the control group or the experimental group, the levels of cortisol in all female tigers after transportation were in good agreement with that before transportation, indicating that the fluctuation of cortisol was not large.

The control group of 156 individuals, despite increased cortisol levels but did not show significant differences, indicating that the stress response of the individual stimuli on the environment is weak, can be quickly restored to the state before the environmental changes (see table 1); the experimental group 993 individuals, although after transport and transport of cortisol before the average the level of fall, but it can be found from the fig. is the largest in the transportation due to stimulation of stress, but can quickly adapt to this change as before quickly returned to the state and as No. 156 (see fig. 2). No. 993 transport in addition to the first cortisol increased significantly, after the overall level of cortisol is lower than that of before transportation, which is likely to be in when it was sampled before the transportation of some environmental factors such as stimulation, and next to the cage in the tiger conflict, or in the breeding process and breeder conflict etc. (see fig. 2).

The control group and the experimental group compared with the female tiger before transportation in the transportation after cortisol levels are increased, the control group increased significantly (P<0.05) and the

experimental group did not significantly increase, the vitamin B6 based compound vitamin B added to reduce the transportation to bring the stress response of female tiger has obvious effect. No matter in the control group or the experimental group, the levels of cortisol in all female tigers after transportation were in good agreement with that before transportation, indicating that the cortisol hormone fluctuation is not as likely as that of male tiger. The levels of cortisol in females 260, 1006 and 1164 were significantly higher than those in other individuals, and even reached 4-6 times the concentration before 149, indicating that the cortisol levels among different individuals were quite different (see table 2, fig. 3 and 4). In addition, the highest level of cortisol was measured for the first time after the transport of the male tiger, indicating that the female tiger had the greatest stress on the day of transportation and then declined gradually.

Compared with fig. 3 and fig. 4, it is not difficult to find that compared with the experimental group, the control group has the largest increase in cortisol level after transportation. This indicates that the stress produced by the female tiger is the largest on the transport day, and the effect of compound vitamin B is also more obvious in the initial stage of anti stress.

No matter in the control group or in the experimental group, male or female, most of the individuals had the highest cortisol level at the beginning of transportation, and declined as time went on (see fig. 1-4). It shows that the stress of the tiger at the beginning of the transfer and transportation is the greatest, and is declining over time.

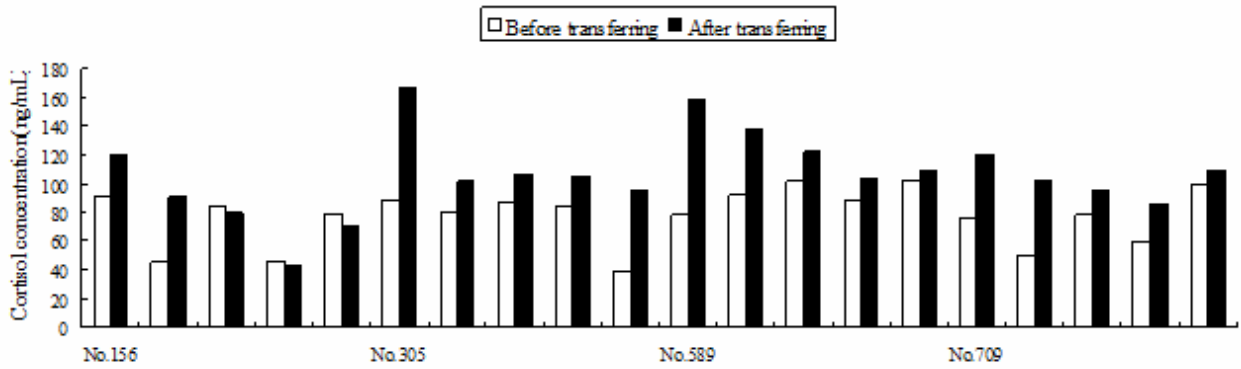


Fig. 1: The contrast and change of cortisol levels in the urine of the male tigers in the control group (ng/mL)

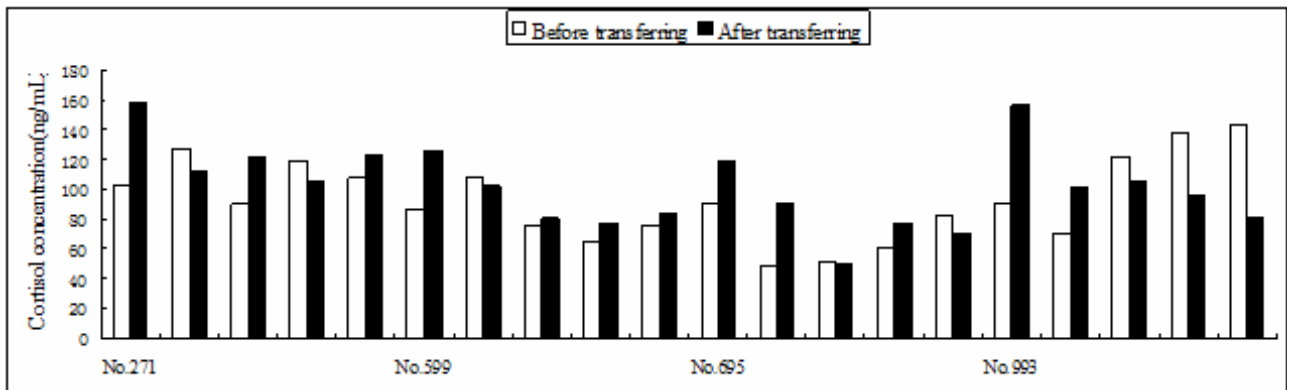


Fig. 2: The contrast and change of cortisol levels in the urine of the male tigers in the experimental group (ng/mL)

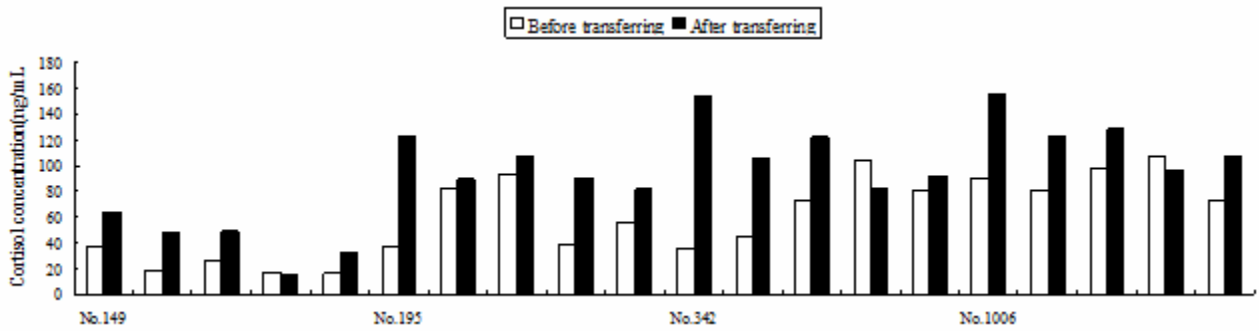


Fig. 3: The contrast and change of cortisol level in the urine of the female tiger in the control group (ng/mL)

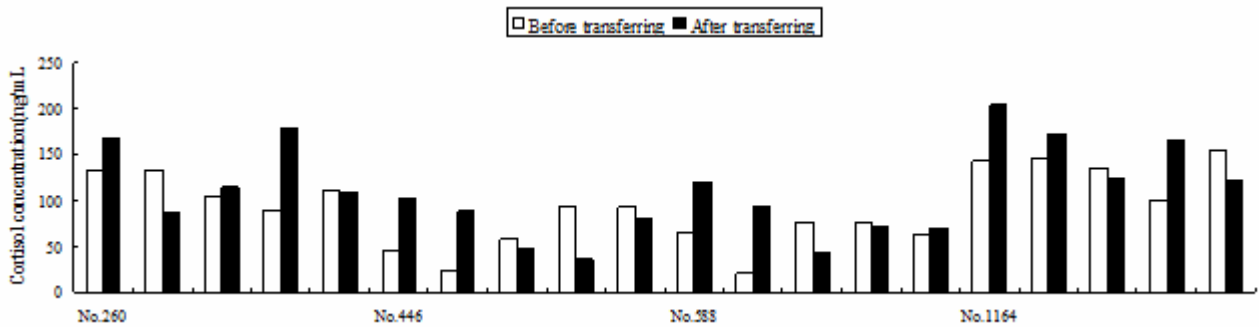


Fig. 4: The contrast and change of cortisol levels in the urine of the female tigers in the experimental group (ng/mL)

Table 1 and 2 by contrast can be found, a female tiger and a male tiger than the control group, after transport and transport before the cortisol levels change compared to more obvious, not only all showed a significant difference, and there were 2 significant differences; in the experimental group female cortisol levels after transport than male fluctuation and these are described more easily than male female tiger by transport stress stimulation.

DISCUSSION

Both the control group and the experimental group showed higher cortisol level (Johnson *et al.*, 1993). The control group had no significant difference in the level of cortisol before transportation compared with that before transportation ($P < 0.01$). This suggests that the levels of cortisol may have a relatively obvious fluctuation in a short period of time, but at the same time it may also be a hormone that is not very volatile (Jessup, 1993; Zhang, 1998; Sun *et al.*, 2015). This may be a protective mechanism formed during the evolution of organisms to prevent the possible harm of excessive stress to the body Yang *et al.*, 1999; Wu *et al.*, 2006; Shi *et al.*, 2015).

The side effects of vitamins are much smaller than those of common drugs, especially sedative drugs, so their effects are widely used in biology (Jianxu *et al.*, 2008; Jin, 2010). Using the sedative drugs compatibility vitamin B6 based compound vitamin B on anti stress effect of the animal can be applied to animal breeding in those susceptible to seizures (Ostojic *et al.*, 2015; Zhang, 2015), a very easy conflict with the environment of the individual, in order to reduce the secretion of cortisol; can also be applied to animal breeding, such as animal mating of those susceptible to environmental interference and affect the normal mating individual use of compound vitamin B can help make mating activities more smoothly (Helin, 1999; Pan *et al.*, 2011), so as to improve the management effect and the female conception; in addition, some labor is more sensitive to the environment of the animal, may be vulnerable to environmental stimuli lead to abnormal childbirth (Paccez *et al.*, 2014), resulting in stillbirth or postpartum abandoned Tsai, not breastfeeding, if timely to add some vitamin B will help to improve animal anti stress, reduce the adverse consequences caused by stress.

CONCLUSION

The family contains more vitamins, in addition to five kinds of compound vitamin B contains other vitamin B and their role on animals remains to be further studied, in order to apply to real life, for animal feed, even human beings bring greater benefits. The urine of two groups before and after transportation was collected, and the content of cortisol in urine was measured by radioimmunoassay as a indicator of stress. Finally,

according to the level of cortisol before and after transportation, the effect of vitamin B6 combined with other B vitamins on the stress resistance of captive Amur tiger was compared between the control group and the experimental group. The results showed that: in most control groups, the levels of cortisol before and after transportation were significantly different ($P < 0.05$), but there was no significant difference in the level of cortisol before and after transportation between male and female tigers in the experimental group ($P > 0.05$). Conclusion: this compound vitamin B, which is mainly composed of sedative and vitamin B6, is obviously helpful for reducing the stress of captive Amur tigers in transferred transportation.

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