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Effects of Exogenous Ghrelin on Feeding, Drinking and Growth Hormone Secretion in African Ostrich Chicks

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ABSTRACT

Background: Ghrelin is widely distributed in an animal's body, playing important biological roles. In mammals, Ghrelin can stimulate food intake and weight gain, but in poultry, it inhibits food intake and drinking. We aimed to determine the effect of Ghrelin on feeding, drinking, body weight and growth hormone (GH) content of African ostrich chicks.

Methods: Sixteen 40-day-old African ostrich chicks were randomly divided into four groups and injected intravenously with saline (control) or Ghrelin (10, 50 and 100 µg/kg) for 7 days. The feeding, drinking and growth hormone (GH) levels using measurement method and radioimmunoassay were determined.

Result: The results show that exogenous Ghrelin increased the feed intake rate, the drinking rate and the secretion of GH in the ostriches.

Key words: African ostrich, Feeding, Ghrelin, Drinking, Growth hormone.

INTRODUCTION

There are many factors that affect the growth and development of poultry, such as Ovo Ornithine-α-Ketoglutarate (Feng *et al.* 2019), Sea Buckthorn (Singh *et al.* 2020), Semi-intensive (Kaya *et al.* 2017). In our experiment, we want to explore whether exogenous Gherlin has an effect on the growth and development of African ostriches. Ghrelin is a polypeptide comprising 28 amino acids that can regulate animal food intake through central regulation or peripheral stimulation (Song *et al.* 2019; Abtahi *et al.* 2017). Studies have demonstrated that central or peripheral injections of Ghrelin can stimulate mammalian food intake and weight gain (Hyland *et al.* 2020; Lee *et al.* 2017; Okuhara *et al.* 2018). However, in poultry, Ghrelin's role is not consistent with the results in mammals (Zhang *et al.* 2020). Injecting mouse Ghrelin into chicks *via* the lateral ventricle inhibited the chicks' feeding (Furuse *et al.* 2001). In the adult Japanese quail, intrapulmonary injection of mouse-derived Ghrelin stimulated food intake. However, when high concentrations of mouse-derived Ghrelin was injected, food intake was inhibited (Shousha *et al.* 2005). Ghrelin promotes growth hormone (GH) release markedly *in vivo* and *in vitro* (Ogawa *et al.* 2018; Yanagi *et al.* 2018; Buntwal *et al.* 2019). Interestingly, injecting non-homologous Ghrelin polypeptides into animals significantly stimulated the release of GH. Preliminary studies in our laboratory have shown that Ghrelin immunopositive cells (Ghrelin-ip) are also widely distributed in many tissues and organs, such as the adrenal gland (Ye *et al.* 2018), pancreas (Wang *et al.* 2017), cerebellum (Wang *et al.* 2012) and gastrointestinal tract (Wang *et al.* 2011) of African ostriches. This distribution is partly similar to that in mammals, which suggested that Ghrelin's physiological role in the growth and development of African ostriches might be similar to that in mammals. There have been no reports on the effects of Ghrelin on the feed and water intake and GH secretion of African ostriches. Therefore, the present research was planned.

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MATERIALS AND METHODS

Seven-day-old African ostrich chicks were obtained from the Ostrich Research Institute of Yangtze University, Hubei, China. The birds had free access to water and the feed was prepared according to the specifications of the Elsenburg Ostrich feed database (Brand, 2010). After 33 days, the birds were weighed and 16 African ostriches chicks (8 females and 8 males) with similar weight (average weight: 3.65±0.53 kg) and growth status were selected for the experiment. The experimental procedures and ostrich treatment procedures have been approved by the Animal Protection and Welfare Committee of our institute.

Experimental procedure

First, the 16 ostriches chicks were divided into four groups (2 male and 2 female ostriches per group) containing four chicks each-one control and three experimental groups. The

ostriches chicks were injected intravenously (metatarsal vein) with saline (control) or ghrelin (experimental) at 08:00 every day for 7 days; the total injection dose was 500 µl per bird. The three experimental groups were treated with 10 µg/kg, 50 µg/kg and 100 µg/kg, respectively, of ostrich ghrelin (synthesized by Shanghai Qiangyao Biological Technology Co., Ltd.) diluted with normal saline. Second, the feed was provided at 08:10, 11:00, 14:00 and 17:00. We re-weighed chow and water 15 min, 30 min, 60 min, 90 min, 120 min, 2.5 h, 3 h, 4 h, 5 h and 7 h after administration and calculated food intake and water consumption. Body weights were observed daily. After 7 days of treatment, the blood was collected from the jugular vein in a vacuum blood collection tube containing EDTA and keep on the ice. It was parted into two parts: one part was stored at -20°C. The other part was utilized to radioimmunoassay.

Measurements of food intake and drinking water

Before each trial of experiment, body weight was measured and the ostrich chicks were distributed into experimental groups so that the average body weight within the groups was as uniform as possible within the same experiment. Ad libitum fed and drinking water ostrich chicks were given a ghrelin injection. Food and water intake were determined by the disappearance of diet and water from the pre-weighted feeder and waterer. Any spillage was also collected and weighed.

Radioimmunoassay

Samples were sent to Beijing Northern Biotechnology Institute for testing (test batch number: 20171213).

Statistical analysis

Results were expressed as means ± SD. Statistical analysis was used analysis of variance statistics software (SAS Institute 2000) with the T-test where appropriate. Values for $p \leq 0.05$ were considered significant.

RESULTS AND DISCUSSION

All results presented are those obtained for both the female and male chicks; no gender-specific effects were observed.

The effect of exogenous Ghrelin on feed intake in African ostrich chicks

Exogenous Ghrelin plays an important physiological role in the feeding process of animals. In the present study, at 15 min after the injection of different doses of Ghrelin, the feed intake of African ostrich chicks decreased. At 30 min after the injection of Ghrelin, there was no change in food intake. At 60 and 120 min after the injection of Ghrelin, food intake increased. At 90 min after the injection, the 10 µg/kg dose group increased, but the 50 µg/kg and 100 µg/kg dose group decreased. In general, there was no significant difference compared with that of the control at 15, 30, 60, 90, 120 min after the injection (Fig 1) ($P>0.05$). At 2.5 h after the injection, the food intake of the 10 µg/kg dose group decreased ($P>0.05$). In the 50 µg/kg dose group, the food intake decreased and the difference was significant ($P<0.05$). In the 100 µg/kg dose group, the food intake increased significantly ($P<0.05$). At 3, 4 and 7 h after Ghrelin injection, the food intake increased in the 10 µg/kg and 50 µg/kg dose groups (both $P>0.05$). However, in the 100 µg/kg dose group, the food intake increased significantly ($P<0.05$). At 5 hours after the injection of Ghrelin, the food intake increased significantly in the 10 µg/kg, 50 µg/kg and 100 µg/kg dose groups (all $P<0.05$) (Fig 2). At 7 days after injection, the total food intake was also tested and the results showed that in the 10 µg/kg, 50 µg/kg and 100 µg/kg dose groups, the food intake of the birds increased significantly (all $P<0.05$) (Fig 3). These results suggested that Ghrelin displays species differences in regulating animal feeding and its regulatory effect in the ostrich is comparable to that of mammals.

The effect of exogenous Ghrelin on drinking water consumption by African ostrich chicks

Ghrelin not only affects the feeding of animals but also influences animal's drinking water consumption (Donald *et al.* 2017).

At 15 minutes after the injection of different doses of Ghrelin, the amount of drinking water consumed by the

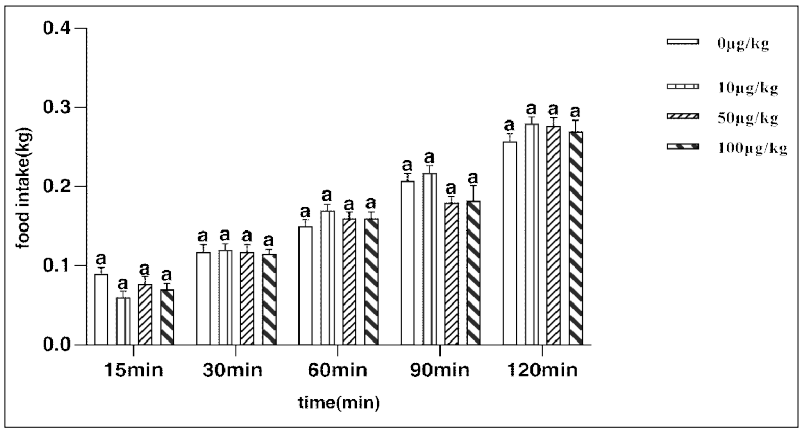

Fig 1: The effect of exogenous Ghrelin on food intake in African ostrich chicks at different times. The histogram shows the different Ghrelin levels on the food intake of the birds at different times. [a] Same letters within the same column indicate no significant differences between the regions.

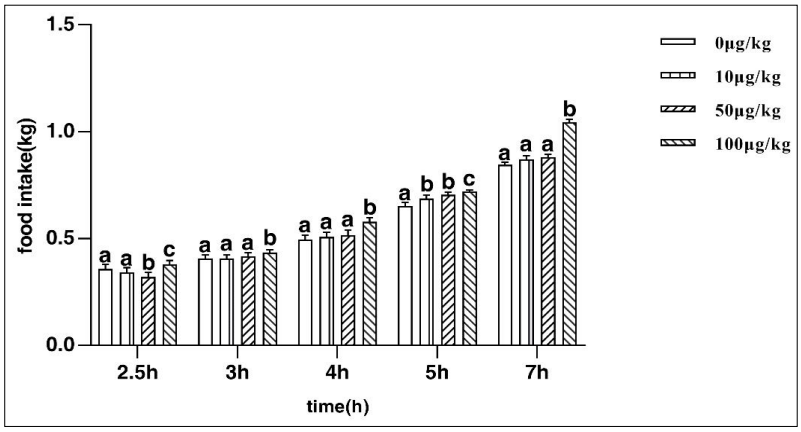

Fig 2: The effect of exogenous Ghrelin on food intake in African ostrich chicks at different times. The histogram shows the different Ghrelin levels on the food intake of the birds in a different time. [a-c]Different letters within the same column indicate significant differences between the regions ($p<0.05$).

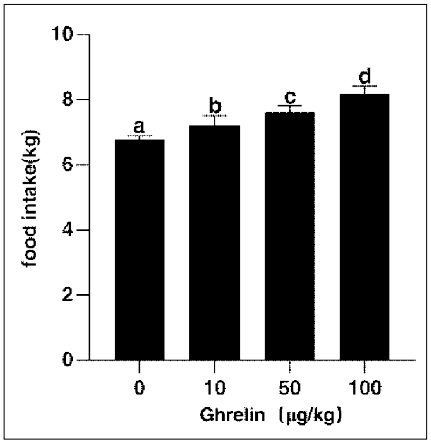

Fig 3: The effect of exogenous Ghrelin on food intake in African Ostrich Chicks at different times. The histogram shows the different Ghrelin levels on the food intake of the birds. [a-d] Different letters within the same column indicate significant differences between the regions ($p<0.05$).

10 μg/kg dose group decreased significantly ($P<0.05$). But the amount of drinking water consumed by the 50 μg/kg and 100 μg/kg dose groups increased (both $P<0.05$). At 30, 60, 90 and 120 min after the injection of different doses of Ghrelin, the water intake of the African ostrich chicks increased; however, the difference in the 10 μg/kg dose group was not significant ($P>0.05$). There was a significant difference in drinking water consumption in the 50 μg/kg and 100 μg/kg dose groups (both $P<0.05$) (Fig 4). At 2.5, 3, 4, 5 and 7 h after the injection of different doses of Ghrelin, the water consumption of the 10 μg/kg dose group decreased ($P>0.05$). However, the amount of drinking water consumed by the 50 μg/kg and 100 μg/kg dose groups increased significantly (both $P<0.05$) (Fig 5). At 7 days of injection, the total drinking water consumption was tested. In the 10 μg/kg dose group, the total water consumption decreased ($P>0.05$). In 50 μg/kg and 100 μg/kg dose groups, the water consumption increased significantly with increasing dose and the difference was significant ($P<0.05$) (Fig 6). These results also suggested that Ghrelin has

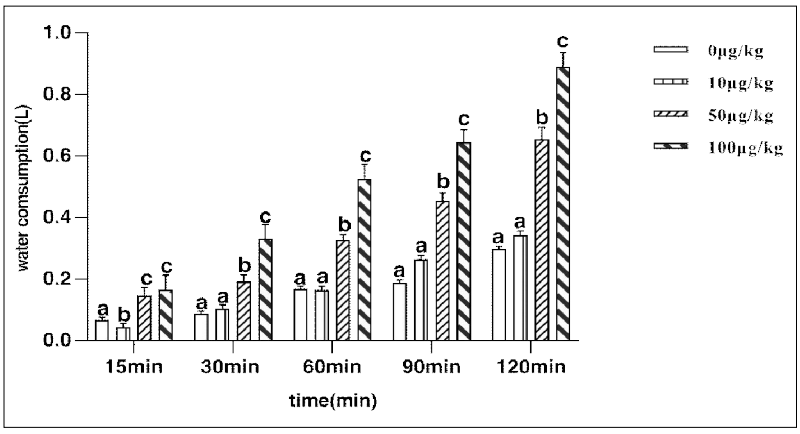

Fig 4: The effect of exogenous Ghrelin on drinking water consumption by African ostrich chicks. The histogram shows the different Ghrelin levels in the drinking water of the birds in a different time. [a-c] Different letters within the same column indicate significant differences between the regions ($p<0.05$).

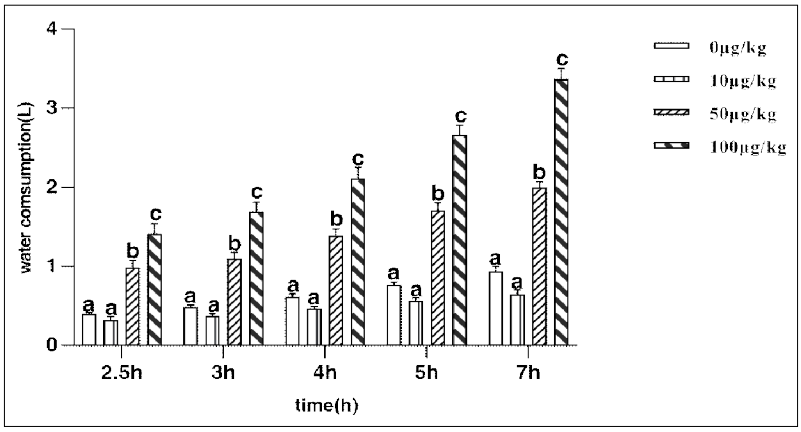

Fig 5: The effect of exogenous Ghrelin on drinking water consumption by African Ostrich chicks. The histogram shows the different Ghrelin levels in the drinking water of the birds in a different time. [a-c]Different letters within the same column indicate significant differences between the regions ($p<0.05$).

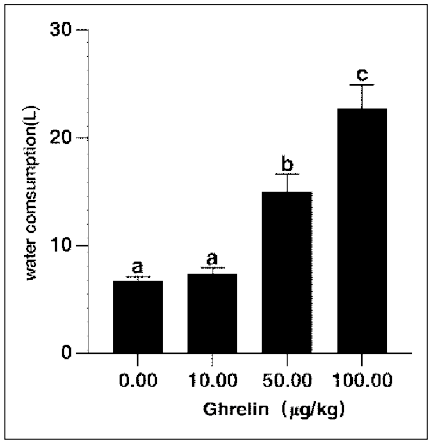

Fig 6: The effect of exogenous Ghrelin on drinking water consumption by African Ostrich Chicks. The histogram shows the different Ghrelin levels in the drinking water for the birds. [a-c] Different letters within the same column indicate significant differences between the regions ($p<0.05$).

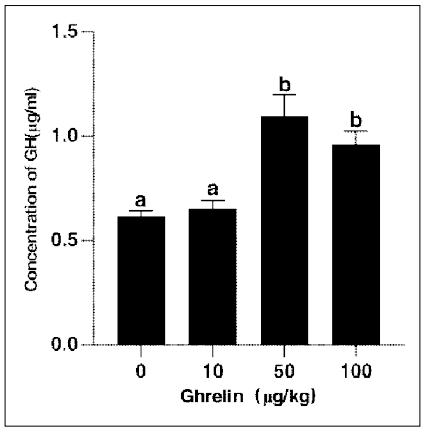

Fig 7: The effect of exogenous Ghrelin on the GH content in African ostrich chicks. The histogram shows the different Ghrelin levels on the content of GH in the plasma of African ostriches. [a-b] Different letters within the same column indicate significant differences between the regions ($p<0.05$).

species differences in regulating animal drinking water intake and its regulatory effect in the ostrich is different from that in other birds. The effect of central injection of ostrich-derived Ghrelin on the drinking water intake of African young ostriches remains be determined.

The effect of exogenous Ghrelin on the GH content in African ostrich chicks

Studies have shown that Ghrelin has a strong GH release promoting effect *in vivo* and *in vitro* (Buntwal *et al.* 2019). In our study, in the 10 μg/kg dose group, the GH content increased, but the difference was not significant ($P>0.05$). By contrast, in the 50 μg/kg and 100 μg/kg dose groups, the GH content increased significantly (both $P<0.05$). The highest GH content was observed in the 50 μg/kg dose group, followed by the 100 μg/kg dose group and 10 μg/kg dose group (Fig 7). Some researchers found that in hypoglycemia, the body's GH response was not regulated by circulating Ghrelin (Flanagan *et al.* 2003). This might be caused by species differences and the animal's physiological conditions, *i.e.*, Ghrelin might have different stimulating effects on GH in different animals and under different physiological conditions. The results of our study showed that Ghrelin could enhance the release of GH from African young ostriches, which also proved that Ghrelin could promote the secretion of GH in birds as well as in mammals.

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Conflict of interest: None.

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