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Effects of Exogenous Ghrelin on Duodenal Growth and Development of African Ostrich Chicks

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ABSTRACT

Background: The African ostrich is the largest herbivorous bird in the world, which has high economic value. However, the brooding period of African ostrich is long. During this period, the various organs of the body are not fully developed, the resistance is low and it is easy to get sick, thus affecting the growth and development. Therefore, it is of great significance to enhance the digestion and absorption capacity of ostrich chicks and improve its growth speed in shortening the incubation period of ostrich, improving the survival rate of ostrich and the economic benefits of ostrich breeding.

Methods: Sixteen 40-day-old African ostrich chicks (male or female) were randomly divided into four groups and injected intravenously of Metatarsal vein with saline (control) or ghrelin (10, 50 and 100 μg/kg) for 6 days. The morphology, gastrin and pepsin levels in the duodenum were measured using stereology, HE staining and radioimmunoassay.

Result: The 10, 50 and 100 μg/kg of ghrelin administered groups showed lower duodenal length and length index than the control group ($P<0.05$). Villus length increased in the 50 μg/kg and 100 μg/kg ghrelin administered groups ($P<0.05$). Crypt depth decreased in the 10 μg/kg ghrelin administered group ($P<0.05$). The ratio of villus length to crypt depth in each group increased and the difference was significant in the 50 μg/kg and 100 μg/kg ghrelin administered groups ($P<0.05$). Pepsin content in the duodenum slightly increased in the 10 μg/kg ghrelin administered group ($P>0.05$) and it significantly decreased in the 50 μg/kg and 100 μg/kg ghrelin administered groups ($P<0.05$). Gastrin content in the duodenum increased significantly ($P<0.05$) in the 10 μg/kg ghrelin administered group and decreased in the 100 μg/kg ghrelin administered group ($P<0.05$).

Key words: African ostrich, Development, Duodenum, Ghrelin, Growth.

INTRODUCTION

Ghrelin is a novel endogenous natural ligand for the growth hormone secretagogue receptor, which is mainly produced by X/A-like cells in the mucosal layer of the gastrointestinal tract (Kojima *et al.* 1999). A small amount of ghrelin is also produced by other organs (Okuhara *et al.* 2018). Ghrelin has been found to be widely expressed in other tissues and organs of animals (Chen *et al.* 2017). A large number of studies have confirmed the inference that the wide distribution of ghrelin immunopositive cells in animals may have many biological roles (Cardona *et al.* 2012). Ghrelin immunopositive cells are widely distributed in the African ostrich (Wang *et al.* 2017a; Wang *et al.* 2017b; Ye *et al.* 2018; Zhang *et al.* 2018), Wang *et al.* (2009) showed that ghrelin immunopositive cells are mainly distributed in the mucosal layer of the gastrointestinal tract of the African ostrich chicks and the number of immunopositive cells gradually decreases from the stomach to the small intestine and then to the large intestine. The number of ghrelin immunopositive cells in the small intestine also showed significant age-related changes, peaked at 90 days and then remained stable. This suggests that ghrelin plays an important physiological role in the digestive system of African ostriches. Previous studies have shown that ghrelin had stimulatory effects on the motility of the antrum and duodenum in both fed and fasted animals (Fujimiya *et al.* 2011). Moreover, Ghrelin can enhance the autophagy of intestinal epithelial cells in rats with sepsis and protect the small intestinal epithelium against sepsis-induced injury (Wan *et al.* 2016). To our knowledge, the effects of exogenous ghrelin on the duodenal growth and development of African ostrich chicks have not yet been reported. Therefore, in this study, we injected different doses of ghrelin into African ostrich chicks *via* the metatarsal vein to investigate the effects of the different doses on the length,

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tissue structure and relevant hormones and enzymes of the duodenum of the ostrich chicks and provide a morphological basis for elucidating the effects of ghrelin on the self-renewal of small intestinal epithelial cells of the chicks. Furthermore, we wanted to provide new ideas for further research on the growth and development of the small intestine of ostrich chicks.

MATERIALS AND METHODS

Animals

The present study was conducted at the Ostrich Institute of Yangtze University in Jingzhou, Hubei in the year of 2018-19. 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 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 (male or female) 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.

Tissue preparation

The 16 ostriches chicks were divided into four groups 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 8 am every day for 6 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. After 6 days of treatment, the birds were weighed and anesthetized with 20% uratan (1 g/kg; China Caoyang No. 2 Middle School Chemical Factory). The abdominal cavity of each bird was opened, The duodenum and its contents were quickly removed, the weight and duodenal length were measured and the length index was calculated. Next, each duodenum is divided into two parts: one part was washed with physiological saline, made into a homogenate and centrifuged (12000 rpm for 10 min) at 4°C and the supernatant was collected. Then, the contents of pepsin and gastrin were measured by radioimmunoassay. The other part was fixed in 4% paraformaldehyde solution and then embedded in paraffin; the paraffin blocks were sectioned (4 µm) using a Leica microtome (Nussloch Gmbh, Wetzlar, Germany) and the sections were used for hematoxylin and eosin (HE) staining.

Morphometric analysis

For each intestinal tissue sample, 3 cross-sections were prepared after the samples had been stained with hematoxylin. Further, for each intestinal cross-section, 10 intact, well-oriented crypt-villus units were selected for experiments conducted in triplicate (30 measurements for each sample). Villus height was measured from the tip to the crypt junction and the crypt depth was calculated as the depth of the invagination between and beside villi using a calibrated ocular micrometer. The ratio of villus/crypt length was also calculated. All the measurements were performed under an Olympus light microscope, using the HMIAS-2000 high-definition chromatic color medical science figure analysis program (Qianping, Wuhan, China).

Statistical analysis

Results are expressed as means ± standard errors on the mean (means ± S.E.). Statistical analysis was done using analysis of variance statistics software with Duncan's multiple range test where appropriate. Differences of $P<0.05$ were considered significant.

RESULTS AND DISCUSSION

Effects of exogenous ghrelin on the duodenum morphology of African ostrich chicks

The proximal part of the small intestine, *i.e.*, the duodenum, receives pancreatic juice and bile, which is mixed with partially digested food from the stomach; it plays a vital role in food processing, both physiologically and chemically (Zhang *et al.* 2020). In the present study, we found that an increase in the body weight of the African ostrich chicks after the injection of 50 µg/kg and 100 µg/kg concentrations of ghrelin ($P>0.05$; Table 1) and the length and length index of the duodenum in the 10 µg/kg, 50 µg/kg and 100 µg/kg groups were significantly lower than those of the control group ($P<0.05$; Table 1) and the 100 µg/kg group yielded the lowest result. Slupecka *et al*. 2012 also found that different doses of ghrelin significantly decreased the length of the duodenum and middle part of the small jejunum. We studied the villus length and crypt depth of the duodenum. The data obtained in this study show that the villus length in the 10 µg/kg group was shorter than that in the control group ($P>0.05$). The villus length increased significantly in both 50 µg/kg and 100 µg/kg groups ($P<0.05$; Table 2, Fig 1) and the higher the villi and their densities, the larger the surface area for digestion and absorption. (Ohanaka *et al.* 2018). In addition, we observed that the

Table 1: Effects of exogenous ghrelin on the length of duodenum of African ostrich chicks.

Exogenous ghrelin (ug/kg)	Body weight (kg)	Length (cm)	Length index (cm/kg)
0	6.3±1.51[a]	78.6±4.66[a]	12.48±2.04[a]
10	6.1±2.41[a]	59.9±5.36[b]	9.82±1.87[b]
50	6.7±1.52[a]	60.9±4.66[b]	9.08±2.08[b]
100	7.1±1.22[a]	48.1±4.23[c]	6.76±1.35[c]

The data is expressed as mean±standard deviation (n=4). [a-c]Different letters in the same column indicate significant differences ($P<0.05$).

crypt depth was slightly reduced in the 50 µg/kg and 100 µg/kg groups when compared with the control group (P>0.05); the crypt depth in the 10 µg/kg group was significantly reduced (P<0.05; Table 2, Fig 1). A large number of stem cells in the crypt can differentiate into intestinal villi cells (Xia *et al.* 2004) and the rate of crypt cell division is revealed by changes in crypt depth that ultimately can influence the process of digestion in the small intestine (Yang *et al.* 2009). The villus length to crypt depth ratio represents the absorption capacity of the small intestine (Liu *et al.* 2020); higher the ratio, stronger the absorption and transport capacity of intestinal epithelial cells (Xie *et al.* 2020). In the current study, we found that, after injection of different concentrations of ghrelin, the ratio of villus length/crypt depth between each dose group increased when compared with the control group; however, it was not significant in the 10 µg/kg group (P>0.05) and the 50 µg/kg and 100 µg/kg groups showed significant differences (P<0.05; Table 2, Fig 1). These findings indicate that exogenous ghrelin can promote crypt differentiation in the duodenum and growth of intestinal villi, thereby improving the absorption of nutrients in the duodenum.

Effects of exogenous ghrelin on digestive enzymes in the duodenum of African ostrich chicks

The chyme of the glandular stomach and muscular stomach of poultry was mainly affected by pepsin and gastric lipase and pepsin was the main enzyme (Zhang *et al.* 2005). Due to the faster passage of foods through the proventriculus and muscular stomach, the digestive effects of pepsin are not strong and protein is mainly digested in the small intestine (Fan, 2003). In this study, pepsin content was higher in the duodenum of the 10 µg/kg group than in that of the control group, but the difference was not significant (P>0.05; Table 3); pepsin content was significantly lower in the 50 µg/kg and 100 µg/kg groups (P<0.05; Table 3). This showed that a high dose of ghrelin inhibited pepsin secretion in the duodenum, whereas a low dose of ghrelin might promoted pepsin secretion. Gastrin is the main hormone responsible for the stimulation of gastric acid secretion. The main biological activity of gastrin is to promote the secretion of gastric acid and pepsin, promote the growth of

Table 2: Effects of exogenous ghrelin on the histology of duodenum of African ostrich chicks.

Exogenous ghrelin (ug/kg)	Villus height (µm)	Crypt depth (µm)	Villus height/ Crypt depth
0	2730.19±300.52[a]	174.64±32.51[a]	16.18±1.04[a]
10	2428.41±215.3[a]	145.01±24.98[b]	16.78±1.25[a]
50	2941.25±278.35[b]	167.69±29.56[a]	17.73±1.09[b]
100	3491.68±298.68[c]	170.55±32.13[a]	21.01±2.02[c]

The data is expressed as mean±standard deviation (n=4). [a-c]Different letters in the same column indicate significant differences (P<0.05).

Table 3: Effect of exogenous ghrelin on pepsin content in duodenum of African ostrich chicks.

Exogenous ghrelin (ug/kg)	Pepsin content (U/ml)
0	5.1±0.2[a]
10	5.3±0.2[a]
50	4.4±0.2[b]
100	4.3±0.1[b]

The data is expressed as mean±standard deviation (n=4). [a-b]Different letters in the same column indicate significant differences (P<0.05).

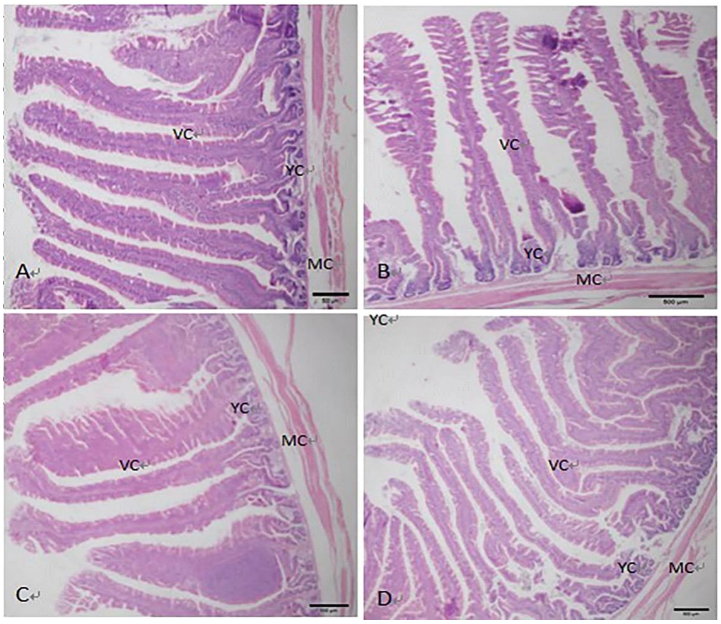

Fig 1: Microstructure of the duodenum of African ostrich chicks with different doses of ghrelin (HE staining). (A) 0 µg/kg dose group (control group). (B) 10 µg/kg dose group. (C) 50 µg/kg dose group. (D) 100 µg/kg dose group. VC, villi; YC, Crypt; MC, Muscularis. Scale bar: 500 µm.

Table 4: Effect of exogenous ghrelin on gastrin content in duodenum of African ostrich chicks.

Exogenous ghrelin (ug/kg)	Gastrin content (U/ml)
0	25.7±1.8[a]
10	78.7±4.6[b]
50	26.3±2.3[a]
100	22.3±1.5[c]

The data is expressed as mean±standard deviation (n=4). [a-c]Different letters in the same column indicate significant differences (P<0.05).

gastrointestinal mucosa, contract the gastric antrum and pyloric sphincter and delay gastric emptying (Liu *et al.* 2012). In this study, gastrin content was significantly higher in the 10 μg/kg group than in the control group (P<0.05; Table 4). Gastrin content was higher in the 50 μg/kg group than in the control group, but the difference was not significant (P>0.05; Table 4). Gastrin content was significantly lower in the 100 μg/kg group than in the control group (P<0.05; Table 4). This showed that a low dose of ghrelin promoted gastrin secretion and a high dose of ghrelin inhibited gastrin secretion. The above results show that a low dose of ghrelin may promote the growth of duodenum mucosa in African ostrich chicks and a high dose of ghrelin may inhibit the growth of duodenum mucosa in African ostrich chicks; however, the specific effects and mechanism need to be studied further.

CONCLUSION

The results of the present study indicated that the development of the duodenum of African ostriches chicks was closely associated with the concentration of ghrelin. The high doses of ghrelin promote duodenal villus growth in African ostrich chicks, but inhibit duodenal length and length index and pepsin and gastrin levels. The low doses of ghrelin inhibit duodenal length and length index, but promote the villus length to crypt depth ratio and gastrin and pepsin levels increased in varying degrees. These data suggest that high doses of ghrelin may inhibit the growth and development of the duodenum of African ostriches chicks, while low doses of ghrelin may promote the growth of the duodenum of ostriches chicks.

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