Physiol. Res. 40: 333–337, 1991

Influence of Hypergravity on the pH Profile and Proteolytic Activity of the Avian Gastrointestinal Tract

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Received April 28, 1990
Accepted October 23, 1990

Summary

The present study deals with the effect of hypergravity (*2xg*) on the pH and on the proteolytic activity in the digesta of the gastrointestinal tract of Japanese quails during intense growth. The birds were raised on a semisynthetic diet containing free amino acids (A) and a commercial diet (B). During days 35 till 40 post-hatching the quails were exposed to hypergravity (*2xg*) using a specially designed centrifuge. On days 40 (experimental group, *2xg*) and 41 (control group, *1xg*) the animals were sacrificed. The pH of the digesta in various segments of the gastrointestinal tract was measured by means of a semi-microelectrode. Total proteolytic activity was determined by means of azo-dye-modified proteins serving as general proteolytic substrates. Hypergravity leads in general to an alkalization of digesta in various parts of the gastrointestinal tract. In case of the gizzard and duodenum (diet A) and also in the distal jejunum (diet B) the differences are significant. With both diets, hypergravity leads to a considerable decrease in the total proteolytic activity. The reduction is most expressed in the duodenum and jejunum. Changes in the pH of digesta compensate for the decrease in the proteolytic activity. This may explain why hypergravity *per se* does not seem to impair growth of the Japanese quails.

Key words:
Japanese quail – Hypergravity – Digesta – pH profile – Proteolytic activity – Space flight

Introduction

Avian species are expected to play an important role in a closed ecological life support system with respect to nitrogen recyclization. Special emphasis is laid on the Japanese quail (*Coturnix coturnix japonica*) in the process of meat and egg protein production from autotrophically grown components and nitrogen-rich by-products such as faeces. While most studies in the field of gravitational physiology deal with mineral metabolism, muscle, the endocrine and immune system (Simmons *et al.* 1990, Mechanic *et al.* 1990, Miu *et al.* 1990, Riley *et al.* 1990, Sapp *et al.* 1990), relatively few studies have been devoted to the influence of changed gravity on gastrointestinal processes. In a very recent study the effect of spaceflight on the proliferation of rat jejunal mucosal cells has been studied (Sawyer *at al.* 1990) with respect to mitotic indices, villus length and crypt depth. No consistent differences between the flight group and controls could be detected. Functional and long-term aspects of hypergravity on the gastrointestinal tract of the Japanese quail have been reported by Otto *et al.* (1988). They observed a slow-down in the intestinal marker transit indicating a decrease in the intestinal propulsion motility. Furthermore, a cranial shift in the site of intestinal lysine absorption in the Japanese quail under the influence of hypergravity was observed. Total lysine absorption was unaffected, however. In this paper the effect of hypergravity (2*xg*) on pH and on proteolytic activity of digesta in the gastrointestinal tract of Japanese quails is described.

Material and Methods

In the experiment male and female Japanese quails (*Coturnix coturnix japonica*) were used. After hatching, birds were assigned randomly to one of two experimental diets A or B. Diet A (Otto *et al.* 1990) is based on wheat-wheat gluten supplemented with synthetic amino acids (protein content 258 g Nx6.25/kg) and diet B is a commercial diet (trade mark KR 1 in Czechoslovakia) containing ground corn, soybean and fish meal (271 g Nx6.25/kg). Animals were raised on a light : dark cycle of 22 : 2 hours, the dark period being from 3 a.m. till 5 a.m. Food and water was offered *ad libitum*. The design of the centrifuge allows exact measurement of food consumption during the period of centrifugation by means of a balance technique. The temperature was gradually decreased from initially 35 °C to 25 °C. During days 35 till 40 post-hatching, i.e. in the end phase of intense growth, 12 birds of each group had been exposed for 24 hours per day to hypergravity (2*xg*). An equal number of animals had been kept at normal gravity serving as the control group. Animals of similar weight (experimental group: 99.1±9.3 g, control group: 92.2±12.1 g) were selected. Hypergravity was produced by a centrifuge spinning at a rate of 22 rpm. The diameter of the rotating arms was 6 m. On day 40 post-hatching the animals of the hypergravity group were anaesthetized with halothane, the gastrointestinal tract was quickly removed and kept at 4 °C and was divided into the following parts: proventriculus plus gizzard, duodenum, proximal jejunum, distal jejunum, ileum plus colon. Immediately after dissection, the pH was measured in the digesta using a combined semi-microelectrode connected to a pH-meter. In most cases the amount of undiluted digesta was sufficient to cover the surface of the electrode. Then the segments were flushed with ice-cold saline. Saline was added to a final volume of 10 ml. An aliquot (250 μl) was used to measure proteolytic activity in each segment. Measurements were performed at 37 °C using Azocoll (Calbiochem-Behring Company, 1979), or a mixture of azo-proteins (Biogema Company, 1988) as general proteolytic substrates. Experimental details can be found in the instruction leaflets to these products. In the case of stomachs, the pH of the proteolytic assay was 1.5. For all other segments, the pH of the assay was adjusted to 8.0. Results are expressed in arbitrary units. However, identical conditions of measurement allow for a direct comparison between experimental and control groups as well as between different diets. Total proteolytic activity was calculated from the sum of activities found in each segment, taking dilution into account. The control group was treated on day 41 post-hatching in an identical manner.

Statistical evaluation of data was performed by means of Student's t-test. Two independent experiments were performed.

Results

The effect of hypergravity on pH of the digesta of the gastrointestinal tract is shown in Tab. 1. In birds raised on diet A and exposed to hypergravity, a trend to alkalization of the digesta as compared to the control group was observed in most parts of the gastrointestinal tract. The differences in pH were statistically significant ($p < 0.05$) only in the case of the gizzard and duodenum. Similar trends were found in the group raised on diet B. Here significant differences were detected in the distal part of the jejunum.

Table 1
Influence of hypergravity on pH profile of digesta along the gastrointestinal tract of Japanese quails raised on two diets

GIT segment	Diet	Experimental group *2xg*	Control group *1xg*
Gizzard	A	2.98±0.18* (12)	2.45±0.16 (12)
Duodenum		6.59±0.04* (11)	6.41±0.06 (11)
Proximal jejunum		7.00±0.07 (12)	7.10±0.11 (12)
Distal jejunum		7.84±0.08 (12)	7.69±0.06 (11)
Ileum		7.61±0.13 (12)	7.28±0.25 (12)
Gizzard	B	2.77±0.21 (12)	2.83±0.11 (12)
Duodenum		6.59±0.04 (12)	6.50±0.05 (12)
Proximal jejunum		6.98±0.10 (12)	7.07±0.13 (12)
Distal jejunum		7.88±0.06** (12)	7.58±0.05 (11)
Ileum		7.84±0.13 (12)	7.74±0.08 (12)

* $p<0.05$,** $p<0.01$; values are given as x±S.E.M. (n)

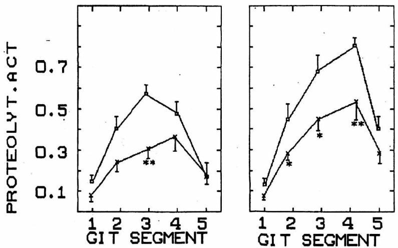

Fig. 1.
Proteolytic activity in the gastrointestinal tract segment (left – diet A, right – diet B). Asterisks – group exposed to hypergravity (*2xg*), squares – control group (*1xg*). The bars indicate S.E.M, (n=12). * $p<0.05$, ** $p<0.01$ – significant differences between experimental and control group.

Another parameter characterizing the influence of hypergravity on digestive processes in the gastrointestinal tract is the proteolytic activity (Fig. 1). The distribution of proteolytic activity along the small intestine was characterized by a well-defined maximum in the jejunum. Differences in the gastrointestinal proteolytic activity between the groups exposed to hypergravity and the corresponding control groups were very clear-cut. With both diets, hypergravity leads to a considerably decreased proteolytic activity in the digesta of the duodenum and jejunum. In some cases, this decrease was highly significant ($p<0.01$). From the data shown in Fig. 1 cumulative values representing the total proteolytic activity of the gastrointestinal tract have been calculated for each group (Tab. 2). Total proteolytic activity in birds exposed to hypergravity was significantly ($p<0.01$) lower than in birds held at normal gravity. The decrease amounted to 41 and 32 % for diets A and B, respectively.

Table 2

Influence of hypergravity on total proteolytic activity in the gastrointestinal tract of Japanese quails raised on two diets

Diet	Experimental group *2xg*	Control group *1xg*
A	1.10±0.14** (11)	1.87±0.15 (11)
B	1.66±0.14** (12)	2.43±0.14 (12)

** $p<0.01$; values are given as $x \pm S.E.M.$ (n)

Discussion

Results presented in this study indicate that moderate hypergravity (*2xg*) may lead to alkalization of the digesta of certain gastrointestinal segments. Changes in the proteolytic activity of individual gastrointestinal segments under the influence of hypergravity are even more profound. Attempts to explain the reason for these changes should comprise the stress factor during centrifugation and subsequent animal handling (Wolf 1981), though the mechanism of stress action on intestinal functions is still not clear (Williams *et al.* 1989). Concerning animal handling, reasonable effort was made to keep all manipulations to a minimum and to treat the control group in exactly the same manner. Recent results by Gažo *et al.* (1988) on the plasma corticosterone level in long-term centrifuged birds do not support the hypothesis that stress is responsible for the observed effects. Another point to be raised is food consumption which, together with saliva, might affect neutralization of gastric acid in the gizzard. However, no significant differences in food consumption between experimental and control groups could be observed for diets A and B (data not shown).

Taking the pH optimum of pepsin and changes of pH and proteolytic activity in the stomach into account, it can be concluded that protein digestion in this part of the gastrointestinal tract will be reduced. In the intestine the situation is more complicated. Here a decrease in proteolytic activity is accompanied by an increase in pH in several segments, the pH being closer to the pH optimum of pancreatic proteases. Obviously such compensation is sufficient to ensure complete digestion of food, since no significant differences in growth and food conversion could be observed between the experimental and control groups (data not shown).

Extrapolation of the results to conditions of microgravity would be rather hazardous at present, although experiments on the effect of hypodynamy on gastrointestinal processes might provide some clues. Such experiments are in progress now.

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