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Digestive Enzyme Activities of Broiler Breeder Pullets Suffering from Stunting Syndrome

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Abstract: The visceral organ weights and activities of pancreatic and intestinal enzymes in a flock of broiler breeder pullets, apparently suffering from stunting syndrome (SS) were compared with those of a healthy flock. Pullets with SS were significantly lighter (P<0.001) than the normal individuals. The relative weights of the pancreas (P<0.05), small intestine (P<0.01) and liver (P<0.01) were higher in the flock with SS than in the normal flock. The specific activities of pancreatic amylase (P<0.001) as well as ileal maltase (P<0.05) and alkaline phosphatase (P<0.001) were increased as a result of SS. The pullets with SS recovered and grew normally again after 16 weeks of age. Some of the symptoms are similar to those described for younger broiler chicks but the results obtained in the current study could be useful in describing SS in older poultry.

Key words: Broiler breeders, pullets, stunting syndrome, enzyme activities

Introduction

Stunting syndrome in broiler chickens is characterized by growth retardation, variability in chick size, leg weakness and diarrhoea (Shapiro and Nir, 1995b; Salyi and Glavits, 1999). The symptoms have been diagnosed in other poultry species but there are conflicting reports on the aetiology. In turkey poults, Ali and Reynolds (1998) have traced the symptoms to an infection with a virus although this was not infectious. In broiler chickens, symptoms have been initiated in healthy flocks through oral inoculation with the mucosa from infected birds, suggesting that the disease can be spread through contact with infected material in feed or water (Shapiro *et al*., 1998). Recently, we encountered a flock of breeder pullets that was apparently infected with SS. This flock failed to grow to the usual standards set by the breeder company.

This study examined the differences between three groups of pullets, from two flocks. Visceral organ weights and the activities of digestive enzymes were evaluated. It is by no means conclusive but attempts to stimulate research towards the establishment of physiological standards for use in diagnosing SS in breeder pullets.

Materials and Methods

It is common practice for broiler breeding companies to have set standards for the growth of chicks from their parent stock. In a particular instance, however, a particular flock failed to attain these standards. Investigations, including those of nutrition and veterinary medicine failed to identify the cause of growth retardation. This study was conducted to compare flocks that were seemingly 'normal' and one flock that was 'abnormal'.

Twelve breeding pullets were obtained from two different flocks. Initially, eight pullets, four from a 'normally' growing flock, 10 weeks old (group 10) and four from a 'poor' flock, 14 weeks old (group 14AB) were sampled. At 14 weeks of age, four pullets were further sampled from the 'normal' flock (group 14N), for more direct comparisons with the 'poor' flock. The pullets were raised on identical diets and physical environment from hatch. The pullets were killed by asphyxiation with CO_2 and weighed. The birds were dissected to obtain the visceral organs, which were weighed. The pancreas and sub-samples of the proximal regions of the jejunum and ileum were collected and snap-frozen in liquid nitrogen for subsequent evaluation of tissue protein content and activities of relevant digestive enzyme activities.

For assessment of digestive enzyme activities and protein content, the pancreas was homogenised in distilled water and centrifuged at 23700 g to obtain a supernatant. The jejunal and ileal tissues were prepared as described by Shirazi-Beechey *et al*. (1991). The tissue was cut into an ice-cold buffer (100 mM mannitol, 2 mM Tris/HEPES, pH 7.1) and the mucosa was then stripped into the buffer using a swirl mixer at high speed for one minute. The mixture was homogenised at medium speed for thirty seconds. Sub-samples of the homogenate were taken into Eppendorf tubes, frozen in liquid nitrogen and stored in a deep freezer (-20 °C) for enzyme analysis, within one week of collection.

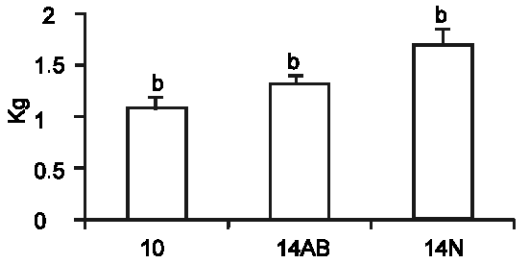
On jejunal and ileal homogenates, biochemical assays were conducted for the activities of maltase (EC. 3.2.1.20) and alkaline phosphatase (AP, EC. 3.1.3.1). For the pancreas, assays were conducted for amylase (EC. 3.2.1.1), lipase (EC. 3.1.1.3) and chymotrypsin (EC. 3.4.21.1). The specific activities of enzymes were measured according to methods previously described for other species (Miller *et al*., 1960; Dahlqvist, 1964; Holdsworth, 1970; Smeltzer *et al*., 1992; Serviere-Zaragoza *et al*., 1997) after standardization for poultry. Assays were conducted at a temperature of 39 °C. For amylase activity, glucose output was measured by incubating with glucose oxidase (Roche Diagnostics, Indianapolis, USA) rather than with dinitrosalicylic acid (DNS) reagent, previously described by Miller *et al*. (1960). The protein content of both the intestinal mucosa and pancreatic tissue was measured according to the method described by Bradford (1976).

Results

The body weight of the 14-week old normal pullets (14N) was significantly higher (P<0.001) than that of the 10-week normal (10) or 14-week old abnormal (14AB) pullets (Fig. 1). The relative weights of the pancreas (P<0.05), small intestine (P<0.01) and liver (P<0.01) were higher in the 14AB pullets than in the other pullets.

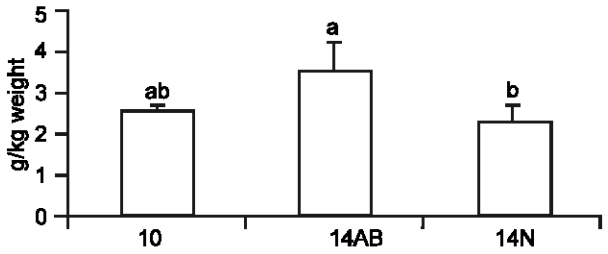
The concentration of protein in the pancreas declined (P<0.001) with age but was significantly higher in the 14AB pullets than the 14N group (Fig. 2). The specific activity of pancreatic amylase was lower (P<0.001) in the 14N group than in the other groups. The concentration of protein in the jejunal mucosa declined (P<0.01) with age (Fig. 3). There was no significant effect of age or growth pattern on the activities of maltase and sucrase in the

a. Body weight



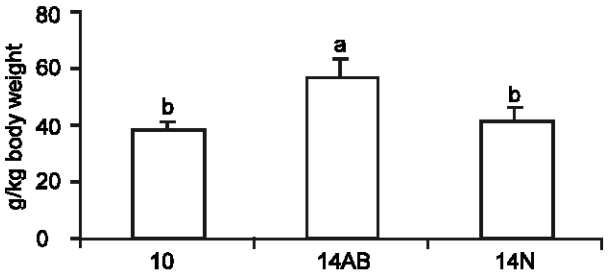
a,b-Mean values not sharing a superscript are significantly (P<0.001) different

b. Pancreas



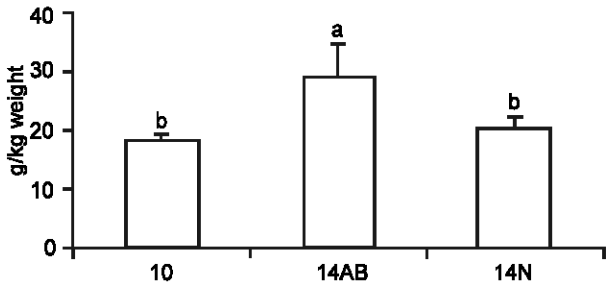
a,b-Mean values not sharing a superscript are significantly (P<0.05) different

c. Small intestine



a,b-Mean values not sharing a superscript are significantly (P<0.01) different

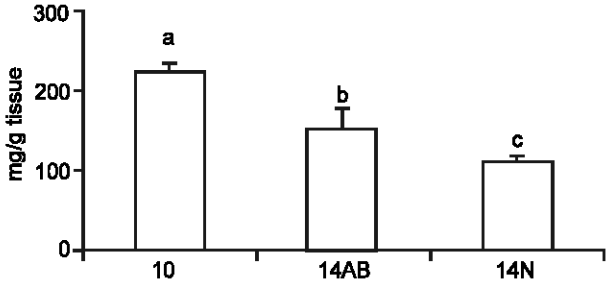
d. Liver



a,b-Mean values not sharing a superscript are significantly (P<0.01) different

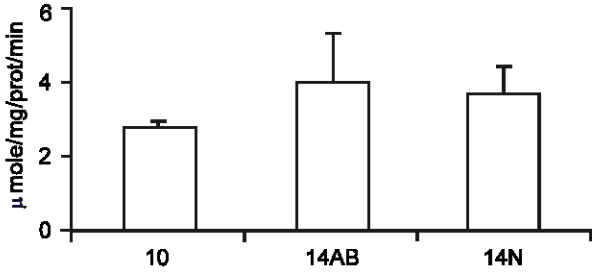
Fig. 1: Body weight and relative weight of visceral organs

a. Protein

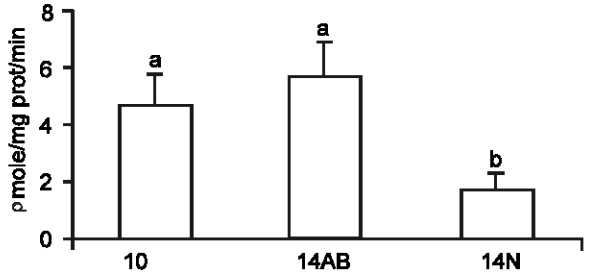


a,b-Mean values not sharing a superscript are significantly (P<0.001) different

b. Chymotrypsin



c. Amylase



a,b-Mean values not sharing a superscript are significantly (P<0.001) different

d. Lipase

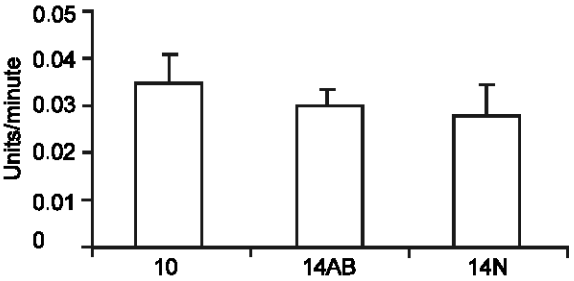
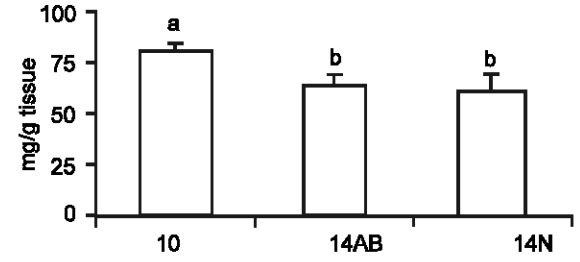


Fig. 2: Concentration of tissue protein and activities of digestive enzymes in the pancreas

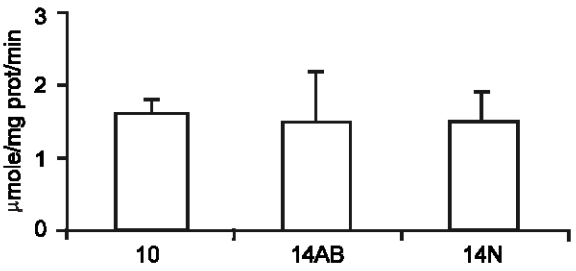
jejunum. In the ileum, mucosal protein content was significantly lower (P<0.001) in the 14AB group than in the other pullets (Fig. 4). There was also a general reduction (P<0.001) in ileal protein content with age. The specific activities of maltase (P<0.05) and alkaline phosphatase (P<0.001) were also higher in the 14AB pullets than in the other two groups but there was no clear trend with age.

a. Protein



a, b-Mean values not sharing a superscript are significantly (P< 0.01) different

b. Maltase



c. Alkaline phosphatase

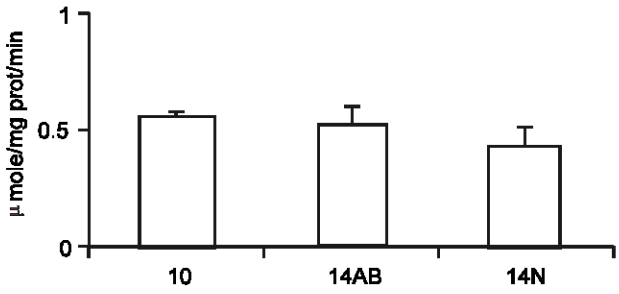
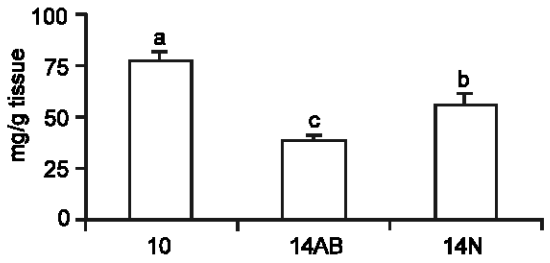


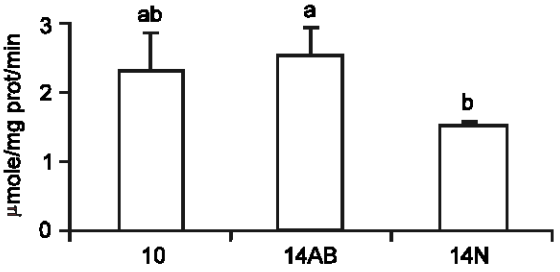
Fig. 3: Concentration of tissue protein and activities of digestive enzymes in jejunal mucosa

a. Protein



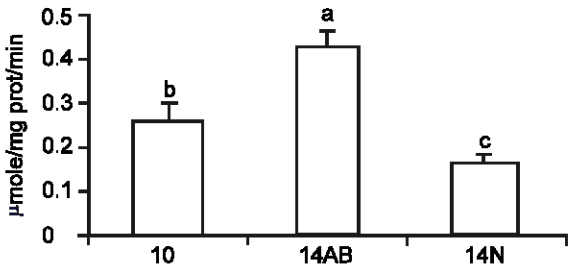
a,b-Mean values not sharing a superscript are significantly (P<0.001) different

b. Maltase



a, b-Mean values not sharing a superscript are significantly (P< 0.05) different

c. Alkaline phosphatase



a, b-Mean values not sharing a superscript are significantly (P< 0.001) different

Fig. 4: Concentration of tissue protein and activities of digestive enzymes in the ileal mucosa

Discussion

The low body weight of the pullets is consistent with observations in meat-type broiler chickens (Shapiro and Nir, 1995b; Salyi and Glavits, 1999). Similar to our observations, Shapiro *et al.* (1998) also reported higher relative weights of the liver and pancreas in broiler chickens infected with SS.

The activities of digestive enzymes in conditions of SS appear to vary with age. In younger (1-2 weeks old) broiler chicks, Shapiro and Nir (1995a) reported a lower activity of trypsin in birds suffering from SS. In 6-week-old broilers, however, the activities of amylase, trypsin and chymotrypsin were higher in inoculated birds than in control birds without SS. In the present study, the activities of chymotrypsin and lipase in the pancreas were, however, similar between the three groups of pullets. Ali and Reynolds (1998) reported a reduction in the activity of jejunal maltase in poults suffering from SS. This was associated with a reduction in the absorption of D-xylose.

It is not certain at what age SS set into the present flock. In meat broiler chicks, Shapiro and Nir (1995b) were unable to stimulate the disease at 14 days of age, when healthy chicks were inoculated with infected material. There have been differences in the pattern of infection between broiler chicks, turkey poults and Leghorn chickens and between ages (Shapiro *et al.*, 1998). While inoculation with the SS agent led to a reduction in the activities of chymotrypsin in 3-week-old broiler chicks, in Leghorns, the activity of the enzyme was increased. It was suggested that Leghorns had a better immune response against the inoculum than did either the broiler chicks or the poults.

Of major interest was the fact that the pullets picked up growth from about 16 weeks of age, without any medical intervention. This spurt of growth may have been due to changes usually experienced at close to maturity. Broiler breeder pullets come into lay at about 24 weeks of age. However, it is similar to the

compensatory growth observed in broiler chickens by Shapiro and Nir (1995b), following initial 4 weeks of symptoms of SS.

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