The effect of heat stress and the use of *Saccharomyces cerevisiae* or (and) bacitracin zinc against heat stress on the intestinal mucosa in quails

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SUMMARY

In this study, determination of the effect of heat stress and use of *Saccharomyces cerevisiae* or (and) bacitracin zinc against heat stress to the height, width of villi and the goblet cell count per unit length of the duodenum, jejunum and ileum in quails was aimed. In this study, 47 56-day old Japanese quails were used. In the serial sections obtained from the intestinal samples the height and widths were measured and the goblet cell count per unit length of the villi determined with the aid of a image analysis program. The height of the villus in the duodenum, jejunum and ileum of the birds exposed to heat stress were shortened and the fall in goblet cell count per unit length in the villi of the ileum was found to be statistically significant whilst that in the duodenum and jejunum were not statistically significant. It was found that in those treated with yeast against heat stress the height of the villi in the jejunum was shortened, in those treated with bacitracin zinc the goblet cell count in the duodenum and jejunum increased, whereas in those treated with yeast-bacitracin zinc the villus height in the jejunum and ileum were found to be shorter with increased goblet cell count. In the combined use of yeast and bacitracin zinc, the individual effects were observed without any synergistic effect found.


Introduction

In quails the comfortable ambient temperature is between 18-30°C. However, with the optimal temperature around 21-27°C, cooling is required when temperatures exceed 30°C [23]. In poultry farms substantial economic losses occur worldwide due to high ambient temperatures. In animals exposed to heat stress an increase in the respiration rate results in respiratory alkalosis and a lowering of the intestinal pH, adhesion or colonization to the mucosa decreasing whilst that of coliform bacteria increases [8].

To reduce the heat stress in poultry provision of clean and cool drinking water, reducing the number of birds per cage, feeding during the cooler times of the day, and addition of electrolyte supplements to their drinking water are being practised [10]. Other measures aimed at reducing the heat stress in these birds include use of vitamins C and E, selenium, [17, 18], antibiotics and probiotics [13, 16, 29] as additives in feeds.

Probiotics are viable microbial additives (like *Lactobacillus acidophilus*, *Streptococcus faecium* and *Saccharomyces cerevisiae*) with the effects of restoring the microbial balance of the gut in the animals they are administered to. They have been reported to inhibit the growth of pathogenic microorganisms and provide digestive enzymes, a desirable effect for the host, and as a result changes in the intestinal microflora, antibiotic production, and synthesis of lactic acid leads to lowering of the intestinal pH, adhesion or colonization to the...
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intestinal mucosa and prevention of ammonium synthesis [9, 12].

The antibiotics used as feed additives, however, have been reported to decrease the microbial production of toxins that suppress growth, reduce the breakdown of essential nutrients in the intestines by microbes, and improvement in the synthesis of vitamin and other growth factors. With this objective antibiotics like bacitracin, oxytetracycline, penicillin are being used [27].

No study on the effect of probiotic and antibiotic use against heat stress on the morphology of the intestinal mucosa in poults was available in the literatures. In this study, the aim was to determine the effects of heat stress and *Saccharomyces cerevisiae* and bacitracin zinc used against heat stress on the height and width of the villi intestinals and the goblet cell count per unit length in the duodenum, jejunum, and ileum.

**Material and Methods**

In the study 47 56-day-old Japanese quails (*Coturnix coturnix japonica*) were used. The birds who were kept in a storey cage system were fed with *ad libitum* feed and water under conventional conditions. During the entire 9-week study period the birds were subjected to a 17-hour daily lightting with the humidity of the experimental rooms kept at 55% ± 5.

In the study, both a control group and 4 treatment groups were formed. The ambient temperature of the control group (n:15) was 20 ± 2°C whilst that of the treatment groups were kept at 32 ± 2°C. During the study period a control group ration (basal diet) containing 20% crude protein, 3000 kcal/kg metabolizable energy and 3% calcium was used. Whereas no material supplement was made to the basal diet supplements were added to the basal diet. The feed supplements in question were prepared weekly in a homogeneous fashion. All groups were fed on these rations for the entire 9 weeks.

After killing of the quails by decapitation tissue samples from the duodenum, jejunum and ileum were obtained. The tissues were then fixed in 10% neutral buffered formalin. Following histological fixation, the tissues were prosessed through a standard alcohol dehydration-xylene sequence and embedded in paraffin. Transversal serial sections (5 \(\mu\)m) were cut at 50 \(\mu\)m intervals from the tissues [21, 24]. The sections were stained by the Crossman’s triple stain for general histological examination and with the periodic acid schiff (PAS) for demonstration of goblet cells in the villi. In each of the six sections taken serially from the tissues, the villus height and width were determined by examining randomly 5 villi and determination of goblet cells done by counting the number of goblet cells in the central 100 \(\mu\)m portion of the 5 villi [2, 4, 26] by means of a image analysis program (Leica Q win standard). Later, the average of 30 values obtained for each animal was taken.

To determine whether or not heat stress has an effect on the goblet cell count, villi height and width in the intestinal mucosa, the t test was used to compare the control group and the first treatment group whilst the ANOVA test was employed on the treatment groups to determine the effect of probiotic and antibiotic administration used against heat stress. Determination of the group source of the difference was done with the Duncan’s test.

**Results**

The average values obtained for the number goblet cells, villi height and width in the intestines for quails fed under normal conditions and those exposed to heat stress are shown in Table I. The goblet cells located on the villi of the duodenum, jejunum, and ileum of those poults exposed to heat stress were noted to be lower than those of the controls. However, this fall though statistically significant in the ileum (P < 0.05) was not significant in the duodenum and jejunum. The width of the villi in the group exposed to heat stress was not found to differ from those of the controls. The villus height in the duodenum, jejunum and ileum of the animals exposed to heat stress was observed to be shorter than those of the control group (P < 0.01, P < 0.001) (Figure 1).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Goblet cells (no./100 (\mu)m)</th>
<th>Villus width ((\mu)m)</th>
<th>Villus height ((\mu)m)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Duodenum</td>
<td>Jejunum</td>
<td>Ileum</td>
</tr>
<tr>
<td>Control</td>
<td>3.97±0.12</td>
<td>6.26±0.18</td>
<td>8.07±0.20</td>
</tr>
<tr>
<td>Heat Stress</td>
<td>3.64±0.08</td>
<td>5.73±0.16</td>
<td>7.31±0.16</td>
</tr>
</tbody>
</table>

| t         | 1.816*   | 1.876*   | 2.458*   | 1.975*   | 0.057*   | 0.722*   | 5.933*** | 3.777*** | 3.025** |

NOTE: In each of the six sections taken serially from the tissues, the villus height and width were determined by examining randomly 5 villi and determination of goblet cells done by counting the number of goblet cells in the central 100 \(\mu\)m portion of the 5 villi by means of an image analysis program. Later, the average of 30 values obtained for each animal was taken.

* : P < 0.05   ** : P < 0.01   *** : P < 0.001   NS : Non-significant

Table I. — Goblet cell numbers, villus width and villus height of small intestine in quails under normal conditions and heat stress conditions (mean ± S.E.).
The average values of goblet cell numbers, villus height and width of the intestines in the quails exposed to heat stress and the feeds of which were supplemented with yeast, bacitracin zinc and yeast-bacitracin zinc rations are shown in Table II. Whereas no difference was observed in the goblet cell number and villus width between those treated with yeast and those treated with the basal diet, the villus height in the jejunum was found to decrease (P < 0.05) (Figure 2) whilst no difference in villus height was observed in the duodenum and ileum. In the group treated with bacitracin zinc, whereas the goblet cell count in the duodenum and jejunum were found to increase relative to the group treated with the basal diet (P < 0.05, P < 0.01) no difference was observed in the ileum. Treatment with bacitracin zinc was found to have no impact on the villus height and width of the intestines. In the group treated with yeast-bacitracin zinc however, the goblet cell count in the jejunum and ileum increased relative to the those fed on the basal diet (P < 0.01, P < 0.05) with no

**TABLE II.** — Goblet cell numbers, villus width and villus height of small intestine in treatment groups (mean ± S.E.).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Duodenum</th>
<th>Jejunum</th>
<th>Ileum</th>
<th>Duodenum</th>
<th>Jejunum</th>
<th>Ileum</th>
<th>Duodenum</th>
<th>Jejunum</th>
<th>Ileum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal diet</td>
<td>3.64±0.08*</td>
<td>5.73±0.10*</td>
<td>7.31±0.16*</td>
<td>151.57±9.25</td>
<td>99.07±7.13</td>
<td>103.08±9.38</td>
<td>930.19±34.72</td>
<td>322.89±27.73*</td>
<td>328.14±14.68*</td>
</tr>
<tr>
<td>Saccharomyces</td>
<td>3.56±0.12a</td>
<td>5.56±0.12c</td>
<td>7.74±0.27a</td>
<td>158.81±8.78</td>
<td>97.46±2.62</td>
<td>102.64±4.30</td>
<td>942.11±42.56</td>
<td>252.61±10.32a</td>
<td>282.76±15.38ab</td>
</tr>
<tr>
<td>cerevisiae Bacitracin Zinc</td>
<td>4.26±0.15b</td>
<td>6.67±0.27b</td>
<td>7.59±0.35a</td>
<td>136.21±5.14</td>
<td>99.01±3.31</td>
<td>97.40±4.33</td>
<td>879.38±67.88</td>
<td>315.74±24.67b</td>
<td>306.11±22.81c</td>
</tr>
<tr>
<td>S. cerevisiae</td>
<td>3.86±0.21a</td>
<td>6.50±0.28b</td>
<td>8.46±0.30a</td>
<td>138.27±4.71</td>
<td>90.19±5.25</td>
<td>98.98±3.59</td>
<td>929.74±29.10</td>
<td>245.97±7.84b</td>
<td>246.69±9.48b</td>
</tr>
<tr>
<td>Bacitracin Zinc</td>
<td>F</td>
<td>2.943*</td>
<td>6.069**</td>
<td>3.060*</td>
<td>2.209ab</td>
<td>0.741ab</td>
<td>0.224ab</td>
<td>0.405ab</td>
<td>4.268*</td>
</tr>
</tbody>
</table>

**NOTE:** In each of the six sections taken serially from the tissues, the villus height and width were determined by examining randomly 5 villi and determination of goblet cells done by counting the number of goblet cells in the central 100 µm portion of the 5 villi by means of a image analysis program. Later, the average of 30 values obtained for each animal was taken.

*: P < 0.05 - **: P < 0.01
a, b, c Means within a column with no common superscript differ significantly. - NS : Non-significant.

*FIGURE 1.* — Photomicrographs of duodenal villi showing. The villus height in the duodenum of quail exposed to heat stress (B) has been shown to be shorter than those of the control group (A). PAS, Bar : 100 µm.

*FIGURE 2.* — Photomicrographs of jejunal villi of quail exposed to heat stress showing. The villus height in the jejunum of quail treated with yeast (B) has been shown to be shorter than those of the fed with basal diet (A). Crossman’s triple stain, Bar : 50 µm.

*FIGURE 3.* — Photomicrographs of ileal villi of quail exposed to heat stress showing. The villus height in the ileum of quail treated with yeast-bacitracin zinc (B) has been shown to be shorter than those of the fed with basal diet (A). PAS, Bar : 50 µm.
change observed in the duodenum. The contribution of yeast- bacitracin zinc did not lead to any differences in the villus width though it led to shortening of villi in the jejunum and ileum (P < 0.05, P < 0.01) (Figure 3) and no change in the height of the duodenal villi.

Discussion

In this study, the villus heights of the duodenum, jejunum and ileum of quails exposed to heat stress were found to be shorter than those of the control group, with the number of goblet cells located in the villi decreasing significantly in the ileum, while that observed in the duodenum and jejunum did not show any statistically significant difference. In our review of the literature no study on the effect of heat stress on the goblet cell counts and the height and width of the villi of the intestines was available. FOX [8] suggested that, in animals exposed to heat stress, the fall in mucin secretion is a result of the increase in corticosteroid hormone secretion, and with mucin as a source of nutrition for anaerobic bacteria, a fall in the number of these bacteria ensues which leads to the increase in coliform bacteria. On the other hand, LEIPER et al., [14] reported that bacterial peptides secreted by nonpathogenic bacteria in the colon mucosa stimulates mucin synthesis and secretion. For this reason, the fall in the goblet cell count in quails exposed to heat stress observed in this study can be interpreted as being a consequence of the effect of corticosteroids which leads to disruption in the microfloral balance. BOLLENGIER-LEE et al., [3] reported that heat stress reduced considerably the feed consumption in chicken. It has been reported that after a 3-day starvation period [22] or withdrawal feedings [24] the height of the intestinal villi of chicken was found to decrease relative to ad libitum feedings. We are of the opinion that the decrease in height of the villi of quails exposed to heat stress observed in this study could be the result of the decrease in feed consumption.

In the group treated with yeast in this study, it was observed that whereas the height of villi shortened only in the jejunum the goblet cells count and width of the villi were found not to differ from those kept on the basal diet. BRADLEY et al., [4] reported a decrease in the number of goblet cells per unit length in the ileum of pouls raised under normal conditions and fed with feeds supplemented with S. cerevisiae. BAUM et al., [2], reported no difference in the goblet cell number between the intestines of swine given supplemented probiotics (Saccharomyces boulardii or Bacillus cereus var Toyoi) during their suckling period and the controls. Without any stress condition, no difference has been reported in the height and width of villi in the small bowel between those fed with probiotic supplemented feed than their controls in chickens [4], man and rats [5]. In studies conducted, it has been reported that under in vitro conditions probiotics increase the levels of short chain fatty acids whilst decreasing the production of ammonium [19]; the short chain fatty acids which are by-products of bacterial fermentation stimulates the proliferation of epithelial cells in the bowels [20]; and that in rats they increase the proliferation of epithelial cells of the bowel [11]. In animals exposed to heat stress no earlier reports on the issue of the effect of supplemented probiotics on the villi height, width, and goblet cell count were available. ZULKIFLI et al., [29] reported higher body weights and much higher increase in weight in chicks treated with Lactobacillus after exposure to heat stress than controls.

In this study the goblet cell number in the duodenum and jejunum of the group treated with bacitracin zinc was found to be higher than those in the group fed on the basal diet whilst no change was seen in the villus height and width. No data on how treatment with bacitracin zinc against heat stress affected the goblet cell count, villus height and width was available. MANNER and WANG [16] in their study on chicken fed with bacitracin zinc supplemented feed against heat stress found that according to the controls the gain in weight, number of eggs and the benefit from feeds were higher, but this difference was not seen when the chicks were fed at 20°C. It has been suggested that under the influence of bacitracin zinc the enterococci count rises as coliform bacteria are inhibited in the bowel and with stimulation of the activities of digestive pancreatic enzymes like amylase, chymotrypsin and lipase [7] an increased the capability of metabolism of the nutrients [15]. In the light of these data, the increase in the goblet cell count observed in this study can be interpreted as being the result of the positive effect of bacitracin zinc administration on the bowel flora as well as the facilitation of absorption of nutrients leading to a decrease in the effect of stress.

In the group treated with yeast-bacitracin zinc in this study, an increase in the goblet cell number in the jejunum and ileum was observed while the villus heights decreased. ABDULRAHIM et al., [1] reported that the effect of a combination of Lactobacillus acidophilus and bacitracin zinc yielded a higher effect on weight gain and benefit from feed much more than their individual effects. No study involving the use of probiotic and antibiotic combination against heat stress was available.

In conclusion, it was found that the heights of villi in the duodenum, jejunum and ileum of quails exposed to heat stress decreased, with the decrease in the number of goblet cells located in the ileal villi found to be statistically significant whilst that of the duodenum and jejunum showed no statistic significance. It was also determined that in those treated with yeast against heat stress, the height of the villi in the jejunum decreased, whilst in those treated with bacitracin zinc the height of the villi in the jejunum and ileum decreased and the goblet cell count increased. In the combined use of yeast and bacitracin zinc only the individual effects, without any synergistic effects, were observed.

References


