INTRODUCTION

Ghrelin, a growth hormone-releasing peptide, was first isolated from the stomach of rats, while searching for an endogenous ligand to an “orphan” G-protein-coupled receptor (Kojima et al., 1999). Subsequently, ghrelin was isolated from chicken proventriculus (Kaiya et al., 2002), and the preproghrelin gene was characterized in turkeys (Richards et al., 2006).

In mammals, ghrelin is a 28-amino acid peptide predominantly produced in the stomach and induces increased feed intake in all species studied to date. Systemic and intracerebroventricular (ICV) administration of ghrelin induces adiposity in rodents by stimulating an acute increase in feed intake as well as a reduction in fat utilization (Tschöp et al., 2000; Hayashida et al., 2001; Wren et al., 2001). Preprandial rise in plasma ghrelin have been observed in humans (Cummings et al., 2001), cattle (Hayashida et al., 2001), rodents (Tschöp et al., 2000), and pigs (Barretero-Hernandez et al., 2010). In addition, active immunization against ghrelin is associated with decreased voluntary feed intake in rodents (Zorrilla et al., 2006) and pigs (Vizcarra et al., 2007).

Active immunization is a procedure that can be used to influence physiological activity by inducing antibodies that neutralize the biological effect of the body’s own hormones. Active immunization against different peptides has been successfully used by us and others in mammalian species (Bowen et al., 2006; Vizcarra et al., 2007, 2012) and avian species (Vizcarra et al., 2001; Avital-Cohen et al., 2011). In essence, “self” antigens are presented to the immune system in the form of conjugates. These conjugates are obtained after a chemical linkage of the antigen to a foreign carrier protein. After primary and booster injections, the autoimmune response is enhanced and endogenously secreted antibodies are produced. A related technique is passive immunization. Passive immunization consists of the transfer of active immunity in the form of readymade antibodies. Passive immunizations have been successfully used in turkeys.

The effect of passive immunization against ghrelin on feed and water intake in turkeys

J. A. Vizcarra, H. Wright, and A. Vizcarra

Food and Animal Sciences, Alabama A&M University, Normal, AL 35762

ABSTRACT

Five-week-old turkeys were used to evaluate the effect of passive immunization against ghrelin on feed and water intake and animal behavior. In experiment 1, females were reared using normal feeding and lighting management recommended by the industry. At 5 wk of age (d 0 of experiment 1), birds (n = 40) were individually caged (0.65 × 0.4 × 0.4 m) with free access to feed and water. Feed and water intake were measured 3 times a day (0800, 1200, and 1700 h) by recording the weight of feed or water offered minus any unconsumed feed or water remaining. After 3 d of adaptation to the cages (d 3), birds were stratified by BW and feed consumption and randomly assigned to a 2 × 5 factorial arrangement of treatment. Starting on d 3, turkeys were given intravenous (iv) injections (0.5, 1.0, 2.0, 4.0, or 8.0 mL) of pooled undiluted plasma obtained from pigs that were previously actively immunized against ghrelin or iv injections (0.5, 1.0, 2.0, 4.0, or 8.0 mL) of pooled undiluted plasma, obtained from nonimmunized pigs (control). In experiment 2, the 2 highest doses (i.e., 4.0 and 8.0 mL; n = 4/treatment) were repeated in a 2 × 2 factorial arrangement as described in experiment 1. A laptop computer with a built-in color camera and appropriate software was used to record birds for 9 consecutive hours, starting 4 h before treatments were applied. Video clips were saved and a human observer watched and annotated bird behavior associated with feeding, drinking, and standing. Passively immunized birds increased feed consumption (P = 0.04) compared with control animals. Water intake was not affected by treatments. There was a tendency for immunized birds to increase the number of pecks per hour and the amount of time devoted for feeding. Our data suggest that in turkeys, the effect of immunization against ghrelin on feed intake is the opposite of that observed in mammalian species.

Key words: ghrelin, passive immunization, feed intake, turkey

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2Corresponding author: jorge.vizcarra@aaumu.edu
In contrast to mammals, significantly less is known on the effect of ghrelin on feed intake in turkeys and other avian species. In general, ICV infusion of ghrelin in chickens and Japanese quail inhibits feed intake (Furse et al., 2001; Saito et al., 2002; Shousha et al., 2005; Xu et al., 2011). However, peripheral administration of ghrelin stimulated feed intake in Japanese quail (Shousha et al., 2005) and had no significant effect on feed intake in layer chickens (Kaiya et al., 2007). In turkeys, ghrelin is predicted to be a 28-amino acid peptide that shares the same 7 amino acids at the N-terminal with that of mammalian origin (Richards et al., 2006). However, to the best of our knowledge, there is no information available on the effect of ghrelin on feed intake in turkeys. Therefore, the objective of this research was to evaluate the effect of passive immunization against ghrelin on feed and water intake in young turkeys.

**MATERIALS AND METHODS**

**Active Immunization**

Active immunization against the first 10 amino acid sequence of ghrelin (N-terminal segment), that contains the common avian-mammalian active core (GSS(octanoid)F) was performed in pigs as previously described (Vizcarra et al., 2007). Briefly, ghrelin was conjugated to BSA by the carbodiimide reaction. The conjugate was emulsified in Freund’s incomplete adjuvant (FIA; Fisher Scientific, Hampton, NH) and diethylaminoethyl-dextran (DEAE; Fisher Scientific). Primary and booster immunizations were given at 19, 22, and 25 wk of age (WOA). Antibody titers against ghrelin were quantified in individual plasma samples and, for reference purposes, they are also reported here. At 32 WOA, pigs were exsanguinated, and a relative large volume of plasma (approximately 0.5 L) was pooled from immunized and control (nonimmunized) animals. Antibody titers (1:100 dilution) for pooled samples were evaluated as previously described (Vizcarra et al., 2007).

**Passive Immunization**

**Experiment 1.** Dose-response studies were performed to evaluate the effect of passive immunization on feed and water consumption in turkeys (*Meleagris gallopavo*). One-day-old broad-breasted white turkeys were reared using normal feeding and lighting management recommended by the industry. Birds were fed a standard corn-soybean-based commercial diet (Alabama Farmers Cooperative, Decatur, AL) that contained 27.0% crude protein, 3.0% crude fat, 5.0% crude fiber, 1.25% calcium, 0.9% phosphorous, and supplemental vitamins and minerals. At 5 WOA (d 0 of the experiment), birds (n = 40) were weighted and individually caged (0.65 × 0.4 × 0.4 m) with free access to feed and water. Feed and water intake was measured 3 times a day (0800, 1200, and 1700 h) by recording the weight of feed or water offered minus any unconsumed feed or water remaining. For statistical analysis purposes (see below), data was expressed as feed and water intake per hour. After 3 d of adaptation to the cages (d 3), birds were weighed and stratified by BW and feed consumption and randomly assigned to a 2 × 5 factorial arrangement of treatment (n = 4/treatment). Starting on d 3 (at 1200 h), turkeys were given intravenous injections (0.5, 1.0, 2.0, 4.0, or 8.0 mL) of pooled undiluted plasma obtained from pigs actively immunized against ghrelin (IMM) or intravenous injections (0.5, 1.0, 2.0, 4.0, or 8.0 mL) of pooled undiluted plasma, obtained from nonimmunized pigs (CTRL). Feed and water intake were measured from d 0 to 4 (end of the experiment).

**Experiment 2.** Anaphylaxis reactions were not evident in experiment 1 at any of the doses used. Therefore, the 2 highest doses (4.0 and 8.0 mL) were repeated using additional birds. Turkeys (n = 4/treatment) were used in a 2 × 2 factorial arrangement as described in experiment 1. A laptop computer with a built-in color camera and appropriate software was used to record birds for 9 consecutive hours, starting 4 h before treatments were applied. The software included a digital timer, with the capability to pause, rewind, and the use of fast and slow motion. Video clips were saved and a human observer watched and annotated bird behavior manually. The following behaviors were recorded: 1. feeding, 2. drinking, and 3. standing. The duration of feeding events (feeding time) was defined as the time that each bird spent with its head in the through. Results are presented as minutes of feeding time per hour. Additionally, the number of pecks from the cup feeder during feeding events was recorded. Results are presented as the number of feeding pecks per hour. The duration of drinking events (drinking time) was defined as the time that each bird spent with its head in the drinking cup. Results are presented as minutes of drinking time per hour. Drinking behavior was also evaluated by counting the apparent ingestion (number of pecks) of water from the cup during drinking events. Results are presented as the number of drinking pecks per hour. Standing behavior (standing) was defined as the time that each bird spent in a standing position that were neither eating nor drinking. Bird behavior data was uploaded into an Excel spreadsheet (Microsoft Corp., Redmond, WA) to summarize data for statistical analyses. The care, treatment, and experimental protocols were approved by Institutional Animal Care and Use Committee of Alabama A&M University.

**Statistical Analysis**

Effects of treatment on feed and water intake (experiment 1) and bird behavior (experiment 2) after
treatments were applied were analyzed using repeated measurements over time (Proc MIXED; SAS Inst. Inc., Cary, NC). The cage was considered the experimental unit, and at least 3 covariance structures were evaluated (compound symmetric, unstructured, and autoregressive). The autoregressive structure provided the best model-fit criteria for all the variables. The model for experiment 1 included the treatment effect (passive immunization or control); the dose (0.5, 1, 2, 4, or 8 mL); the time of sampling (1700 on d 3; 0800 and 1200 on d 4), and the interactions. The model for behavioral data in experiment 2 included the treatment effect (passive immunization or control); the dose (4 or 8 mL), and the interaction. Behavioral data was not normally distributed and was log(e)-transformed for statistical analysis purposes. To evaluate possible differences between treatments before treatments were applied (i.e., d 3), BW, feed and water intake, and bird behavior were analyzed using a one-way ANOVA test.

RESULTS

Active Immunization

Two weeks after primary immunization, the percentage of bound $^{125}$I-ghrelin in plasma from immunized pigs was significantly increased compared with control animals (Figure 1; open symbols). Antibody titers for pooled samples obtained at 35 WOA were 39.5% and 0.01% for immunized and control animals, respectively (Figure 1; closed symbols).

Passive Immunization

Experiment 1. No significant differences were observed in feed and water intake and BW before treatments were applied between immunized and nonimmunized birds. After treatments were applied, there was a significant immunization effect on feed intake. However, there was not a dose or time effect, and there was no interaction of dose or time with immunization. Therefore, pooled means for immunizations (across doses and time) are presented in Table 1. Birds that were passively immunized against ghrelin consumed on average 31% more feed than control animals ($P < 0.04$). Water intake was not affected by treatments, doses, or time.

Experiment 2. Bird behavior (feeding, drinking, and standing) was similar for all birds before treatments were applied. After treatments were applied, immunized birds tended to increase feeding time and the number of feeding pecks per hour compared with control birds. There was not a dose effect, and there was not an interaction of dose with immunization. Therefore, pooled means for immunizations (across doses) are presented in Table 1.

DISCUSSION

We successfully collected antibodies from actively immunized pigs that, in turn, were used to passively immunize turkeys. In contrast to mammalian species (Nakazato et al., 2001; Zorrilla et al., 2006; Vizcarra et

Table 1. Least squares means for feed and water intake (experiment 1) and animal behavior (experiment 2) in turkeys that were passively immunized against ghrelin

<table>
<thead>
<tr>
<th>Variable</th>
<th>IMM¹</th>
<th>CTRL²</th>
<th>P-value</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Feed intake (g/h)</td>
<td>6.3</td>
<td>4.8</td>
<td>0.04</td>
<td>0.70</td>
</tr>
<tr>
<td>Water intake (g/h)</td>
<td>16.4</td>
<td>15.1</td>
<td>0.64</td>
<td>1.8</td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feeding time (min)</td>
<td>1.3</td>
<td>0.8</td>
<td>0.13</td>
<td>0.28</td>
</tr>
<tr>
<td>Feeding pecks per h</td>
<td>53.5</td>
<td>33.8</td>
<td>0.11</td>
<td>9.5</td>
</tr>
<tr>
<td>Drinking time (min)</td>
<td>0.8</td>
<td>0.8</td>
<td>0.79</td>
<td>0.22</td>
</tr>
<tr>
<td>Drinking pecks per h</td>
<td>8.1</td>
<td>8.8</td>
<td>0.68</td>
<td>1.1</td>
</tr>
<tr>
<td>Standing time (min)</td>
<td>27.7</td>
<td>29.2</td>
<td>0.57</td>
<td>2.6</td>
</tr>
</tbody>
</table>

¹IMM = passive immunization.
²CTRL = control.
³Pooled least squares means averaged over doses and time (n = 20 per treatment).
⁴Pooled least squares means averaged over doses (n = 8 per treatment).
increased feeding time. Feeding behavior in poultry intake (Table 1).

In addition, ghrelin stimulates hypothalamic AMP-activated protein kinase (AMPK), suggesting that AMPK activation may mediate the orexigenic effect of ghrelin in mammals (Kola, 2008; Kola and Korbonits, 2009). In birds, ghrelin is not associated with the activation of NPY. Infusion (ICV) of ghrelin in 1-d-old chicks increased corticotropin-releasing factor without changes in hypothalamic NPY mRNA expression (Saito et al., 2006). Additionally, infusion of ghrelin (ICV) in neonatal chickens inhibited AMPK gene expression (Xu et al., 2011). Taken together, the differential effect of ghrelin on food intake in birds involves, at least, the activation of different neuropeptides in the central nervous system and the inhibitory effect on AMPK.

Although feed intake was increased in turkeys that were passively immunized against ghrelin, water intake was not affected (Table 1). The effect of ghrelin on water consumption is controversial. Infusion (ICV; 0.01–0.1 nmol) of ghrelin in 4-d-old chickens resulted in a dose- and time-dependent antidiipsogenic effect under ad libitum and water-deprived conditions (Tachibana et al., 2006). Conversely, water consumption was not affected in 5-d-old chickens selected for low and high BW and infused (ICV; 0.1–0.4 nmol) with ghrelin (Xu et al., 2011). In other species, such as rodents (Hashimoto et al., 2007; Mietlicki et al., 2009) and eels (Kozaka et al., 2006), infused (ICV; 0.01–0.4 nmol) with ghrelin potently inhibits water intake in rats (Nakazato et al., 2001). To the best of our knowledge, this is the first report on the effect of passive immunization against ghrelin in poultry. The lack of a dose effect in the present experiment suggests that the antibodies provided at the lowest dose were sufficient to elicit a maximum increase in feed intake.

We concluded that in turkeys, passive immunization against ghrelin significantly increases feed intake. The effect of immunization against ghrelin on feed consumption in turkeys is the opposite of that observed in mammalian species.

**REFERENCES**


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