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Research article

# Effects of dietary macronutrient composition on exogenous neuropeptide Y's stimulation of food intake in chicks

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## HIGHLIGHTS

- Neuropeptide Y increases food intake independent of diet.
- Neuropeptide Y causes preferential protein and carbohydrate intake in chicks.
- High fat feeding increased the magnitude and duration of food intake response to neuropeptide.

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## ABSTRACT

In mammalian models it is well documented that the potent orexigenic factor, neuropeptide Y (NPY) causes preferential intake of high carbohydrate and fat diets; however, information on this is limited in non-mammalian species. The purpose of this study was to determine the influence of dietary macronutrient composition on NPY's orexigenic effect in chicks. Three isocaloric diets were formulated: high carbohydrate, fat and protein. In Experiment 1, chicks were fed the three diets and received intracerebroventricular injections of 0.2 or 2.0 nmol NPY. Chicks that consumed the high carbohydrate and protein diets had a non-dose dependent similar magnitude of increased food intake after NPY injection, but those on the high fat diet had a dose dependent food intake increase. In Experiment 2, when chicks were given free access to all three diets, injection of 0.2 nmol NPY caused preferential increase in intake of only the high protein diet whereas 2.0 nmol NPY caused preferential increases in of both high carbohydrate and protein diets. Neither dose affected high fat diet intake. In Experiment 3, chicks were raised on one of the three diets and then switched to the others. When chicks were raised on the high fat and protein diets and then switched to the other diets, stimulation of food intake occurred for the same duration, 180 min. However, when chicks were raised on the high carbohydrate and then switched to high fat, NPY injection caused a sustaining increase in cumulative food intake that lasted the entire observation period. These results suggest that NPY has selective effects on consumption of carbohydrate, fat and protein in chicks, and that diet in turn affects the NPY-mediated response in food intake, with a high fat diet enhancing NPY sensitivity that is associated with a greater magnitude and duration of feeding response. In turn, NPY caused preferential protein and carbohydrate intake instead of fat intake (in this order of preference), when chicks had the opportunity to select their diet. Finally, these results reinforce that NPY increases food intake independent of diet.

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## 1. Introduction

Diet composition influences appetite and animals will alter intake of a nutrient to meet demands for physiological processes [20,21,25]. The preference of rats for dietary protein was influenced by the protein content of their previous meal, with consumption of a high protein diet leading to a preference for carbohydrates and vice versa [19]. In general, high protein diets induce satiety [8,19], whereas diets low or deficient in protein induce an increase in food intake perhaps as a compensatory response [2,3,33]. With the rise in obesity, it is clear that excess consumption of fats and carbohydrates are linked to compulsive feeding behavior [23].

Neuropeptide Y (NPY), one of the most potent orexigenic neuropeptides in the central nervous system, has been associated with hyperphagia and obesity [14]. NPY is abundant in the hypothalamus and in rodents, central injection of NPY increased carbohydrate

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[22,26,27,32] and fat [9,27] intake, leading to obesity [11,17]. Macronutrient composition in turn influences NPY-mediated food intake. After four weeks of consuming diets that differed in macronutrient content, rats with free-choice access to diets showed increased sensitivity in their food intake response of chow and fat but not sugar to exogenous NPY [30].

There was reduced food intake in chickens fed an isoenergetic high protein (30% crude protein) diet or isonitrogenous diet with an imbalance of amino acids [29]. During the first two weeks post hatch, there was reduced intake in broilers that consumed isocaloric diets with relatively poor quality protein [16], and reduced intake of a diet containing 12% crude protein as compared to 18 or 24 [10]. Less is known about effects of dietary fat on food intake in chickens and to our knowledge reported effects that compare macronutrients during the early post hatch stage are sparse. The majority of studies with chickens used diets that were not isocaloric, confounding effects of dietary protein or fat content with energy density [29]. Moreover, the relationship between dietary macronutrient composition and NPY is not well understood in non-mammalian vertebrates. It was our objective to measure food intake after NPY injection in chicks fed isoenergetic diets that differed in macronutrient composition. This model may provide novel insight into mechanisms of how NPY-mediated food intake is influenced by diet.

## 2. Methods

Hubbard X Cobb 500 day of hatch chicks (commercial broiler) were obtained from a local hatchery and caged individually with 30 ± 2 °C and 50 ± 5% relative humidity with 24 h of light. Chicks were handled daily to adapt to handling and minimize stress, with ad libitum access to diet and tap water. Diets were formulated as shown in Table 1 and mixed at Augusta Cooperative Feed Mill (Staunton, Virginia, USA). The high carbohydrate diet was formulated to meet the minimum requirements defined for the starter phase of commercial broilers (http://www.cobb-vantress.com) and serves as a broiler industry standard starter diet. The high protein diet was formulated to contain 30% crude protein and the high fat diet to have 60% of the metabolizable energy derived from calories in refined lard, which is designed to be similar to a common rodent obesogenic diet [4]. Diets were isocaloric and isonitrogenous and formulated to meet minimum digestible amino acid requirements for commercial chicks. Experimental procedures were performed according to the National Research Council Publication, Guide for Care and Use of Laboratory Animals and were approved by the Virginia Tech Animal Care and Use Committee.

**Table 1**
Ingredient and chemical composition of experimental diets.

| Ingredient (% as-fed)[a] | High carbohydrate | High protein | High fat |
|---|---|---|---|
| Ground corn | 58.80 | 34.64 | 2.16 |
| Soybean meal | 36.12 | 57.48 | 42.48 |
| Soybean hulls | 0.00 | 0.00 | 27.71 |
| Lard | 0.00 | 0.00 | 24.00 |
| Soybean oil | 1.2 | 4.80 | 0.00 |
| Methionine 99% | 0.28 | 0.04 | 0.35 |
| Threonine | 0.10 | 0.00 | 0.07 |
| L-Lysine 78% | 0.09 | 0.00 | 0.00 |
| Dicalcium phosphate | 1.54 | 1.41 | 1.62 |
| Calcium carbonate | 1.15 | 1.07 | 1.01 |
| Sodium bicarbonate | 0.15 | | 0 |
| SALT920831 | 0.37 | 0.36 | 0.37 |
| Coban 90[b] | 0.05 | 0.05 | 0.05 |
| Phytase-RONOZYME[c] | 0.05 | 0.05 | 0.05 |
| Vitamin and mineral premix[d] | 0.10 | 0.10 | 0.10 |
| Choline Chloride-60% | 0.00 | 0.00 | 0.01 |
| Kcal ME/kg | 3,000 | 3000 | 3050 |
| Crude protein | 22% | 30% | 22% |
| Crude fat | 3.7% | 6.7% | 25% |
| Crude fiber | 2.5% | 2.6% | 12.4% |

[a] Diets were formulated to meet or exceed minimum recommended specifications for Cobb-500 broilers during the starter phase (Cobb-Vantress).
[b] Coban 90 (Elanco Animal Health) contains 90 g of Monensin sodium per pound of premix and is included in the diet as a coccidiostat.
[c] DSM Nutritional Products, Ltd.
[d] Guaranteed analysis (per kg of premix): Manganese, 25.6 g; selenium, 120 mg; zinc, 30 g; Vitamin A, 4409, 171.076 IU; Vitamin D3, 1410,934.744 ICU; 13,227.513 IU; d-biotin, 88.183 mg.

Chicks were injected using a method adapted from Davis et al. [12] that does not appear to induce physiological stress [15]. The head of the 4-day post hatch un-anesthetized chick was briefly inserted into a restraining device that left the cranium exposed and allowed for free-hand injection. Injection coordinates were 3 mm anterior to the coronal suture, 1 mm lateral from the sagittal suture, and 2 mm deep targeting the left lateral ventricle. Anatomical landmarks were determined visually and by palpation. Injection depth was controlled by placing a plastic tubing sheath over the needle. The needle remained at injection depth in the un-anesthetized chick for 10 s post injection to reduce backflow following the injection which occurred over the course of 3 s.

All experiments were conducted at 4 days post hatch with a free hand intracerebroventricular (ICV) injection method as described previously [12,24]. Chicken NPY (YPSKPDSPGEDAPAEDMARYYSALRHYINLITRQRY, AnaSpec, San Jose, CA, USA) was custom synthesized and dissolved in avian artificial cerebrospinal fluid [1] and injected at a total volume of 5 μL with 0.06% Evans blue dye to facilitate injection site localization. While it is true a chicken produces 1.4 μL/min cerebrospinal fluid [1], it has been shown that an ICV injection volume up to 10 μL does not affect food intake [13]. At the completion of data collection, chicks were euthanized and their brains dissected to determine accuracy of injection into the lateral ventricle. Any chick without dye present in the lateral ventricle was eliminated from the analysis. Sex was determined visually by dissection.

In Experiment 1, chicks were randomly assigned to one of the three diets at day of hatch, with ad libitum access to food and water. On day 4 post hatch, chicks were randomly assigned one of three ICV NPY doses: 0 (vehicle only), 0.2 or 2.0 nmol, which were administered between 05:00 and 07:00. After ICV injection, chicks were returned to their cages and had ad lib access to both diet and water. Food intake was quantified up to 180 min following injection (Fig. 1). Food intake data were converted to a percentage of body weight by taking food weight consumed divided by the chick's body weight at injection time and multiplying by 100. Experiments were replicated and effect of replicate was not significant, thus data were pooled. Data were analyzed using two-way ANOVA within time point using the GLM procedure of SAS 9.3 (SAS Institute, Cary, NC) and the statistical model included the main effects of treatment and diet and their interaction. The diet by NPY dose was significant and thus secondary ANOVAs were conducted within each diet. In Experiment 3, a student's *t*-test was performed to compare treatments within a time point for each dietary group. Sex was not significant in any experiment and removed from the model. Tukey's method was used post hoc to separate the means. All data are presented as means ± standard error and differences considered significant at $P < 0.05$.

In Experiment 2 procedures were the same as in Experiment 1, except that each chick had access to all the 3 diets prior to and after ICV NPY injection. In Experiment 3 procedures were the same as Experiment 1, except that chicks were raised on one of the 3 diets and then switched to another at the time of 0.2 nmol NPY injection and food intake was recorded up to 360 min following injection.