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Central and peripheral injection of abscisic acid had no effect on food intake and body weight in broiler chicks

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Abstract An important phytohormone, abscisic acid (ABA) regulates primary physiological functions in plants. However, animal tissues including brain especially hypothalamus, hippocampus, cortex, and cerebellum are also known to produce ABA. A three-stage study was conducted to determine the effect of intra-cerebroventricular (ICV) and peripheral injection of ABA on broiler's food intake. The first and second stages were included 5 and 7-day-old chicks that received ICV injection of ABA at doses of 1.25 and 2.5 µg and 5 and 10 µg of ABA respectively. Food intake was measured 1, 2, 3, 6, 12, 21, and 24 hours after injection. During the third stage, chicks were injected daily with 200 µg of ABA subcutaneously (SC) for six days. Food intake and body weight were measured daily. ICV injection of ABA did not affect broilers' food intake compared to control group. In addition, SC injection of ABA for 6 days had no effect on measured traits, except on the 4th day that food intake increased significantly in ABA group. The results of this study showed that ABA did not significantly affect the food consumption in neonatal broiler chicks.

Introduction

Due to differences in environmental cues, habitual feeding patterns, and circadian rhythms, appetite control, and energy homeostasis mechanisms are very different and complex among animals [1]. There is a set of physiological mechanisms that regulate appetite control and food intake, which are attached to different parts of the nervous system. According to some literatures, broiler growth was primarily due to increased food intake, and food quality only played a minor role [2]. Various endogenous and exogenous hormonal and metabolic substances regulate feeding behavior in animals [1].

Abscisic acid (ABA) is an isopronoid plant hormone with a molecular formula of C15H20O4 and a molar mass of 264.32 g mol. researches have shown that there are similarities between ABA in plants and retinoic acid (RA) in animals. Both compounds are made from the precursor of vitamin A (beta-carotene) and also have a free carboxyl group at the end of their side chain, which plays an important role in their biological activity [3]. ABA is found in some vegetables and fruits like figs, blueberries, apricots, bananas, potatoes, soy milk, apples, and olives [4]. The endogenous hormone ABA

can also be found in a wide range of animal cells [5]. Researchers have shown that ABA is produced in different brain regions including hypothalamus, hippocampus, cortex, and cerebellum [5]. In spite of this, the distribution of ABA-producing cells in the central nervous system has not yet been completely elucidated. Target cells are stimulated by ABA by activating LANCL2, a member of the LANCL family which contributes to immunity, inflammation, and metabolism. By binding ABA-LANCL2, cAMP and calcium are increased within the cell, which then triggers the release of immune and pro-inflammatory mediators [6]. Some evidence suggests that ABA plays a role in the regulation of metabolic processes [7].

To our knowledge, there are no experimental data indicating ABA's effectiveness in regulating appetite and body weight in broilers. Although many investigations have been conducted on phytohormones and their biological function in plants, no comprehensive study has been conducted on how they can regulate food intake and energy consumption in poultry, and it is crucial to specify their potential role as a dietary nutrient for feeding and weight control. Enhancing food intake is a major factor in causing broiler growth to increase. The aim of this study was to investigate the effect of intracerebroventricular and subcutaneous injection of ABA on food intake and also subcutaneous injection on body weight in broilers.

Materials and Methods

Animals

One-day-old broiler chicks (Ross 308) were prepared. The birds were initially kept in mass in an automatic controlled cage with free access to water and food. For the first 48 hours, the cage temperature was 33° C and humidity was 55 ±5% and then temperature was reduced to 30° C and humidity to 50 ±5%. The chicks were kept in the continuous lighting.

Ration: The birds' diet (a starter diet containing 21% crude protein and 3200 kcal ME/kg) was constant throughout the experiment and they had free access to water and food at all times except during the three hours of fasting.

ICV injection method:

ICV injection was performed into the right lateral ventricle of chicks, using a stereotaxic device based on the method of Jonaidi et al [8] . The volume of injection was 10 µl of saline solution (0.85% NaCl) and DMSO plus Evans Blue (0.1%) for the control group; and the same solution with the same volume containing the corresponding doses of ABA for the treatment groups (doses of 1.25 and 2.5 µg for 5-day-old chicks and doses of 5 and 10 µg for 7-day-old chicks). Each injection lasted 15 seconds to prevent the solution from escaping from the bird's brain. Since the skulls of 5 and 7-day-old chicks are cartilaginous, the needle easily entered the bird's skull.

Subcutaneously injection method:

An insulin syringe was used for subcutaneous injection. During the injection, the chicks were restrained by hand and then the neck skin was lifted by hand so that the needle head could easily enter the area beneath the skin. Following this, the solution with a volume of 200µl was slowly injected.

Study protocol

In this study, the effects of ICV and SC injection of ABA on food intake in broilers were studied in 3 stages. In the first stage the chicks were kept for 5 days and in the second stage for 7 days. On the day of injection, the chicks were fasted for three hours before injection in order to synchronize their food intake. After ICV injection, the fresh weighed food was given to the chicks and the food intake was measured at 1, 2, 3, 6, 12, 21 and 24 hours post-injection. At the end of the 24-hour period, the chicks were anesthetized with isoflurane and euthanized, their brains were then removed to check the accuracy of the injection site. The data from birds that were not properly injected with the solution were not included in analysis. In the third stage ABA was

injected SC for 6 days. Food intake and body weight were measured daily for six days.

Statistical analysis method:

In order to analyze data and draw the diagrams, Prism software version 9.0.0 (121) was used. Data was analyzed using a two-way analysis of variance (ANOVA) with repeated measure. Significance level was considered $P < 0.05$.

Results

The effect of ICV injection of ABA on food intake in 5-day-old chicks

The effect of ICV injection of ABA at doses of 1.25 and 2.5 µg were compared with the control (vehicle) group. According to the results, the amount of food intake did not significantly change following administration of different doses of ABA on 5-day-old chicks during time phases of test (Fig. 1).

The effect of ICV injection of ABA on food intake in 7-day-old chicks

In this experiment, the effect of ICV injection of ABA at doses of 5 and 10 µg were compared with the control group. The results showed that the doses of 5 and 10 µg had no effect on food intake on chicks compared to the control group (Fig. 2).

The effect of SC injection of ABA on food intake and body weight in 7-day-old chicks

In this experiment, the effect of SC injection of ABA at doses of 200 µg were compared with the control group. The results showed that SC injection of ABA significantly increased food intake on the day 4 compared to the control group. In contrast, there were no significant difference in food intake on the first three days, as well as the fifth and sixth days (Fig. 3). The data analysis of the current study showed that SC injection of 200 µg dose of ABA has not any significant effect on body weight of chicks (Fig. 4).

Discussion

ABA as a phytohormone has oxidative anti-stress activity. Antioxidants in certain concentrations can also play a role in regulating nutritional activities [7]. In this study, the central and peripheral injection of abscisic acid on the appetite of broiler chicks was investigated. ICV injection of different doses of ABA had no effect on food intake in 5 and 7-day-old broiler chicks compared to the control group. In this method of injection, the substance is injected directly into the one of ventricles, so it can reach to the brain tissues without any alteration by peripheral organs. There are many neural cites and complex networks in the brain that are involved in food intake and body weight regulation and among these, hypothalamus plays a critical role on sensing and integration of energy signals relaying from peripheral organs to the brain. Some of these intra and extra hypothalamic cites are responsible for increasing food intake while the others decrease it. Thus, it is difficult to explain how and why ABA could not alter consumption of food and, more investigations are needed in this regard. However, some possible explanations are as below:

In 1986, it was found that the ABA in the rat brain is independent of the ABA in its diet. Indeed, the amount of this hormone in the brain was much higher than in the peripheral tissues [9]. Qi et al reported that the concentration of ABA in the hypothalamus is higher than in other brain tissues [5]. It is possible that this hormone is produced endogenously in chicks in such abundance that it occupies the receptors and exogenous ABA cannot combine with the its receptors.

ABA induces its effects through several signaling pathways, including LANCL2 and PPAR receptors [10]. Among these effects, we can mention interference in the action of hormones

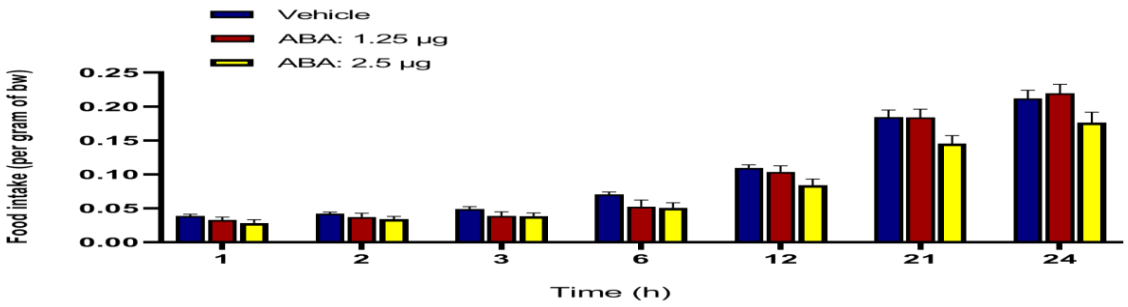

Fig 1: Average cumulative food intake based on body weight. 1.25 µg and 2.5 µg doses of ABA were ICV injected to 5-day-old broiler chicks. All data are presented as mean ± S.E.M. n = 10 for each group.

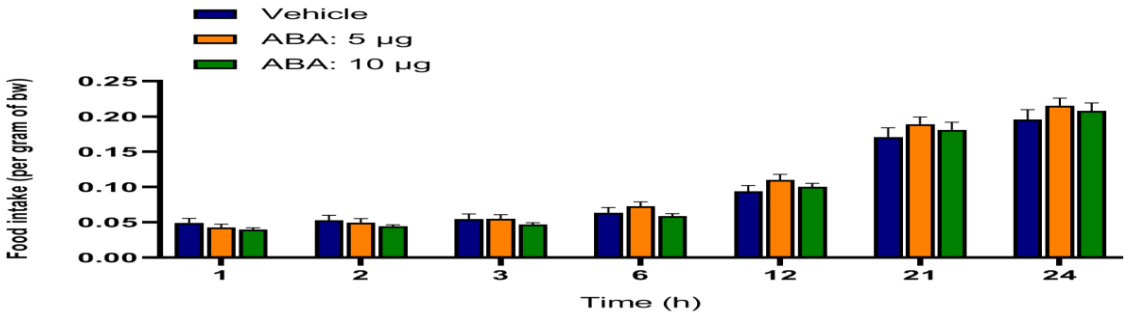

Fig 2: Average cumulative food intake based on body weight. 5 µg and 10 µg doses of ABA were ICV injected to 7-day-old broiler chicks. All data are presented as mean ± S.E.M. n = 11 for control group and 12 for treatment groups.

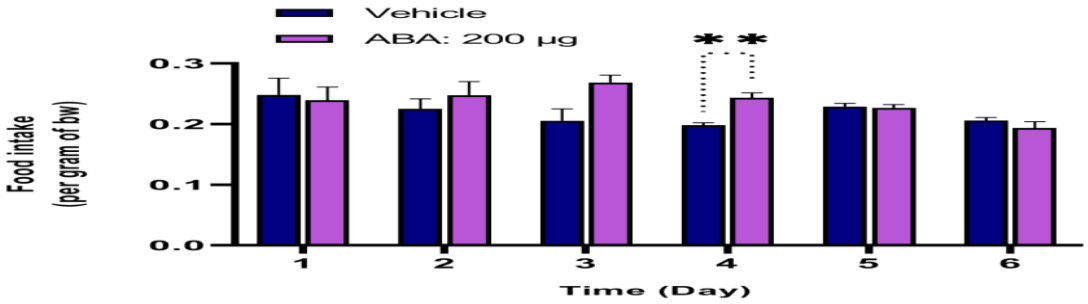

Fig 3: Average cumulative food intake based on body weight. 200 µg dose of ABA was SC injected to broiler chicks from day 8 to 13 chronically. All data are presented as mean ± S.E.M. n = 8 for each group.

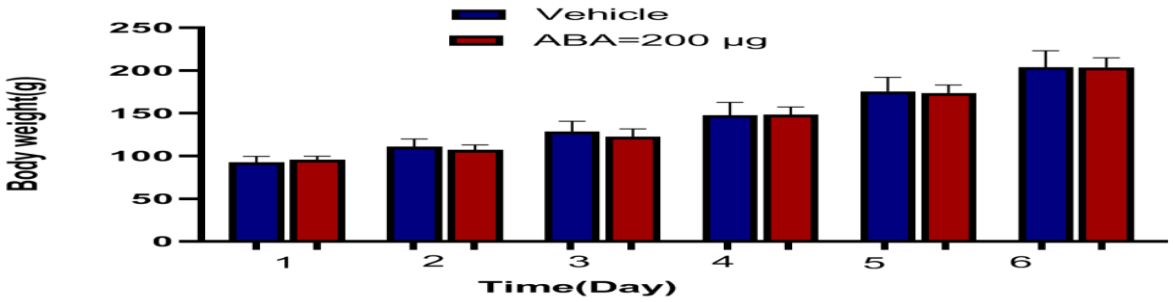

Fig 4: Effect of subcutaneous administration of 200 µg dose of ABA on body weight. ABA was SC injected to broiler chicks from day 8 to 13 chronically. All data are presented as mean ± S.E.M. n = 8 for each group.

and neurotransmitters that control nutrition and metabolism, such as glutamate, GABA and retinoic acid, that all these compounds somehow play a role in controlling nutritional activity and metabolism. In some studies, it has been shown that ICV injection of glutamate reduced food intake in rodents. Also, glutamate has a hypophagic effect in broilers, and this effect is probably carried out by ionotropic receptors [11]. The stimulatory role of GABA in the regulation of feeding behavior has been demonstrated in mammals and birds. ICV administration of the GABA receptor agonist, muscimol, stimulates feeding in rat, pig and broiler [12][13][14]. This response can be blocked by the specific GABA receptor antagonist, bicuculline. In this study, the ineffectiveness of ABA in broiler chicks is probably the result of the conflict between glutamate and GABA. Considering the limited studies conducted in this case as well as the limited findings of this article, it is necessary to investigate these possibilities in the future.

According to the studies, food intake and meal frequency are regulated by different mechanisms. Activation of mesolimbic projections with NAc increases meal frequency without any effect on total food intake [15]. Also, central injection of ABA in rats increased the frequency of meals and decreased the duration of meals, while it had no effect on total food intake [7]. According to the evidence in this study, this possibility can also be in the case of broiler chicks.

Peripheral and chronic injection of ABA significantly increased food intake only on the day 4 after injection. However, repeated measure ANOVA data analysis showed that ABA cannot alter food intake during 5-days period of injections. Despite a lot of research in the past decades, there are still other unknown peripheral signals that play a role in appetite regulation, and ABA is probably one of them and more researches are needed in this field. However, the decrease in appetite in broilers after day 4 in chronic injections is probably due to down regulation.

In birds, insulin is known as a hypophagic hormone that interacts with neuropeptide Y (NPY), pro-epiomelanocortin (POMC) and corticotropin-releasing factor (CRF) in the brain [16]. Probably, ABA caused an increase in insulin expression, which decreased food intake in birds from the day 4 onwards.

In this study chronic peripheral injection of ABA did not affect the body weight of chicks. Body weight regulation is a complex process that is initially regulated by calorie intake and energy consumption. However, body weight is also affected by various physiological and psychological factors that cause changes in energy consumption without food restriction, mainly through modulating the regulatory role of the hypothalamus on the basal metabolic rate [17][18]. In the current study, the extension of the time period of chronic peripheral injection, might have led to change in body weight.

Conclusion

As far as we know, this study was the first research on the effect of ABA in birds. We showed that ICV injections of ABA as a phytohormone at different doses had no effect on food intake in 5 and 7-day-old broilers and also, daily subcutaneous injection of this substance for 5 days had no effect on body weight and food intake with an exception of an increase in food intake only on the fourth day of treatment. More investigation is needed to clarify the exact effect of ABA on the energy balance and metabolism of chicks.

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Conflict of interest

The authors declare that there is no conflict of interest.

Ethical approval

All ethical considerations including utilizing animals were considered cautiously.

References

1. Wynne K, Stanley S, Mcgowan B, Bloom S. Appetite control. 2005;291–318.
2. Richards MP. Mechanisms Regulating Feed Intake , Energy Expenditure , and Body Weight in Poultry 1 , 2. Poult Sci . 86(7):1478–90.DOI: 10.1093/ps/86.7.1478
3. Addicott FT, Lyon JL, Ohkuma K, Thiessen WE, Carns HR, Smith OE, et al. (1968) Abscisic acid: a new name for abscisin II (dormin). Science.159 (3822):1493.
4. Magnone M, Ameri P, Salis A, Andraghetti G, Emionite L, Murialdo G, et al. (2015) Microgram amounts of abscisic acid in fruit extracts improve glucose tolerance and reduce insulinemia in rats and in humans. FASEB J. 4;29 (12):4783–93. DOI: 10.1096/fj.15-277731.
5. Qi CC, Ge JF, Zhou JN. (2015) Preliminary evidence that abscisic acid improves spatial memory in rats. Physiol Behav.139:231–9.DOI: 10.1016/j.physbeh.2014.11.053
6. Sturla L, Fresia C, Guida L, Bruzzone S, Scarfì S, Usai C, et al. (2009) LANCL2 is necessary for abscisic acid binding and signaling in human granulocytes and in rat insulinoma cells. J Biol Chem. 284(41):28045–57.
7. Soti M, Abbasnejad M, Kooshki R, Esmaeili-Mahani S. (2019) Central microinjection of phytohormone abscisic acid changes feeding behavior, decreases body weight, and reduces brain oxidative stress in rats. Nutr Neurosci. 22 (10):678–87.DOI: 10.1080/1028415X.2018.1431093
8. Jonaidi H, Rasooli R. (2013) Effect of central enterostatin on fat intake in neonatal chicks. Neurosci Lett. 533 :60–4.
9. Le Page-Degivry -Th. M, Bidard JN, Rouvier E. (1986) Presence of abscisic acid, a phytohormone, in the mammalian brain. Proc Natl Acad Sci U S A. 83(4):1155–8.
10. Derosa G, Maffioli P, D'angelo A, Preti PS, Tenore G, Novellino E. (2020) Abscisic acid treatment in patients with prediabetes. Nutrients. 1; 12(10):1–11.DOI: 10.3390/nu12102931
11. Zendehdel M, Baghbanzadeh A, Babapour V, Cheraghi J. (2009) The effects of bicuculline and muscimol on glutamate-induced feeding behavior in broiler cockerels. J Comp Physiol A Neuroethol Sensory, Neural, Behav Physiol.195(8):715–20.
12. Kokare DM, Patole AM, Carta A, Chopde CT, Subhedar NK. (2006) GABAA receptors mediate orexin-A induced stimulation of food intake. Neuropharmacology.50(1):16–24.DOI: 10.1016/j.neuropharm.2005.07.019
13. Baldwin BA, Ebenezer IS, De La Riva C. (1990) Effects of intracerebroventricular injection of muscimol or GABA on operant feeding in pigs. Physiol Behav. 48(3):417–21.DOI: 10.1016/0031-9384(90)90337-4
14. Jonaidi H, Babapour V, Denbow DM. (2002) GABAergic control of food intake in the meat-type chickens. 76:465–8.DOI:10.1016/S0031-9384 (02)00692-3
15. Boekhoudt L, Roelofs TJM, De Jong JW, De Leeuw AE, Luijendijk MCM, Wolterink-Donselaar IG, et al. (2017) Does activation of midbrain dopamine neurons promote or reduce feeding? Int J Obes. 1; 41(7):1131–40.
16. Braun EJ, Sweazea KL. (2008) Glucose regulation in birds. Vol. 151, Comparative Biochemistry and Physiology - B Biochemistry and Molecular Biology. Elsevier Inc.p. 1–9.
17. Dossat AM, Lilly N, Kay K, Williams DL. (2011) Glucagon-like peptide 1 receptors in nucleus accumbens affect food intake. J Neurosci. 12; 31(41):14453–7. DOI: 10.1523/JNEUROSCI.3262-11.2011
18. Wang XF, Liu JJ, Xia J, Liu J, Mirabella V, Pang ZP. (2015) Endogenous Glucagon-like Peptide-1 Suppresses High-Fat Food Intake by Reducing Synaptic Drive onto Mesolimbic Dopamine Neurons. Cell Rep. 4;12(5):726–33.DOI: 10.1016/j.celrep.2015.06.062

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