Stimulation of growth and development in Japanese quails after oral administration of ecdysteroid-containing diet

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Abstract. Freshly hatched Japanese quails of the strain Farao were fed for 50 days by diets containing graded amounts of pulverized seeds of an Asiatic plant Leucaea carthamoides Iljin. The dry seeds of this plant contained 1.8%–2.1% of 20-hydroxyecdysone, together with some smaller amounts of other ecdysteroids. In additional experiments the quails were also offered the standard diet plus whole seeds of Leucaea ad libitum and the standard diet enriched with certain commercially available biostimulating preparations for birds, Biostrong and Ecowit.

The effects of the diets were evaluated first at the age of 37 days and then at the age of 50 days, which covered the reproduction period. Investigations related to the effects on growth, behaviour and overall developmental conditions were completed by RIA analysis of ecdysteroid concentrations circulating in the blood of the quails.

The basic experimental series involved testing of the following diets: 0) Standard diet (SD); 1) SD + 0.2% Leucaea seed; 2) SD + 1.0% Leucaea; 3) SD + 5.0% Leucaea; 4) SD + 5.0% Biostrong; 5) SD + 3.0% Ecowit, and; 6) SD + the whole seeds of Leucaea ad libitum. At the age of 37 days we found the following percentage increase of the living mass (Control group = 100%): 1) 102.8%; 2) 109.5%; 3) 120.4%; 4) 101.9%; 5) 104.9%; and 6) 103.5%. These results confirm previous findings of strong anabolic effects of Leucaea in growth of the vertebrate animals. However, since the plant contains a number of other biologically active classes of the secondary plant substances, the true responsibility of ecdysteroids for the described effects cannot be guaranteed, without further studies with pure ecdysteroid. The results of RIA analyses revealed that the amount of 20-hydroxyecdysone circulating in the blood of Japanese quails was directly proportional to the amount of dietary ecdysteroid. The largest concentrations, around 80 ng/ml of blood were found in quails which consumed the whole seeds of Leucaea.

INTRODUCTION

Due to their pronounced effects on growth and proliferation of insect tissues, ecdysone and ecdysteroids have been often suspected of the possibility of having analogous stimulatory effects on growth in the vertebrates. Earlier studies, initiated with 20-hydroxyecdysone in the late 1960, revealed stimulation of protein synthesis, especially in the in vitro preparations from liver and other organs of mice (for more information see Burdette, 1972). During the past two decades, some new data on the effects of ecdysteroids in vertebrates have been published (see review by Simon & Koolman, 1989). Among these effects we can find pronounced anabolic, tonic, or adaptogenic effects caused by oral
administration of certain ecdysteroid-containing plants with the diet of mice or rat (see Syrov, 1984).

In this study we have investigated possible anabolic effects of ecdysteroids, that are contained in large amounts (20 mg per g of dry mass) in the seeds of *Leuzea carthamoides* (Abubakirov, 1984; Baltaev & Abubakirov, 1987) on growth, puberty and reproduction of the Japanese quails (*Coturnix japonica*). The dry seeds used in these experiments contained 1.8 to 2.1% of 20-hydroxyecdysone, with minor amounts of several other ecdysteroids. Japanese quails have been selected due to their fast development, small size, early sexual maturity, and very good egg yields combined with relatively small food consumption (Nitsan, 1992). These and other properties (see Wilson et al., 1961) make the Japanese quail an ideal biological model for future experiments with small amounts of pure ecdysteroids. Although the final aim of this study would involve a detailed analysis of biophysical and biochemical changes in blood and in various organs of these animals after the autopsies, the here presented topic has been limited to the effects of dietary ecdysteroid on growth of the living mass and the content of ecdysteroid in blood serum. For comparison, the quails have been also offered with the diet enriched with certain commercially available biostimulating preparations for birds, Biostrong and Ecovit.

**MATERIALS AND METHODS**

The experiments were performed on 1000 freshly hatched quails of the strain Farao. The young birds were divided into 6 experimental groups, each containing 80 specimens, and 5 control groups, with 100 specimens in each group. In the control group they were fed with the food mixture BR-1 for broilers during 36 days. Since then, they received another food mixture, N-1 which is more suitable for hens (Composition of BR-1: 22.02% protein, 2.98% fat, 3.11% ash, 11.91 MJ of metabolizable energy, 3.54% fiber, 88.78% dry matter proportion; N-1: 17.02% protein, 4.89% fat, 10.20% ash, 3.08% fiber, 10.86 MJ of metabolizable energy, 89.17% dry matter proportion). The experimental specimens received the above diet with the addition of the following ingredients: 0) Standard diet (SD); 1) SD + 0.2% of pulverized *Leuzea* seed; 2) SD + 1.0% of *Leuzea*; 3) SD + 5.0% of *Leuzea*; 4) SD + 5.0% Biostrong 500 (tonic preparation supplied by Delacon Biotechnik, Steyregg, Austria); 5) SD + 3.0% Ecovit (polyfactorial probiotic preparation for poultry, manufactured by Medipharm Hustopeče, Czech Republic); 6) SD + whole seeds of *Leuzea* ad libitum.

The breeding experiments were carried out at optimal zoohygienic conditions, in special breeding cages (STS Hostvice), under 16 h light : 8 h dark phase photoperiod. During the whole developmental period we constantly monitored the growth curves, food conversion index, overall health conditions and behaviour in the separate groups. At the age of 30 and 50 days, a determined part of the experimental quails was sacrificed in order to determine the morphological, metabolic, biochemical and immunological changes between the separate groups.

The radioimmunoassay determination of ecdysteroids in blood serum were made according to the procedures described by Chang & O'Connor (1979), using 20-hydroxyecdysone (Sigma) as a competitive binder and standard. The dried methanolic extracts of blood were dissolved in 50 μl of borate buffer (pH 8.4, with 0.075 M NaCl) containing approx. 5000 dpm of 23,24-3H-ecdysone (NEI, sp. activity 50 Ci/mmol). This solution was mixed with an antiserum H-22 (obtained by the courtesy of Prof. L.J. Gilbert of Northwestern University, USA) in 50 μl of borate buffer. The mixture was incubated overnight at 4°C, the immunoglobulins were precipitated with ammonium sulphate (50% saturation) and resuspended in a toluene-tritron scintillation liquid. Radioactivity was measured with Tri-carb-liquid Scintillation Counter (Packard), the calculations were made using the SecuRia computer programme (Packard). For statistical analysis of the data we used the Student-Newman-Keuls Multiple Comparisons Test.
RESULTS AND DISCUSSION

All the food additives tested were accepted by the quails without any remarkable changes or aversion. The growth curves of the control groups corresponded with the data given for the corresponding age and genetic strain of Japanese quails by Šebová (1988). Major part of the results has been shown in Fig. 1. In general, we have found an increased body mass after 30 or 37 days of the initial breeding period in all experimental groups. In comparison to the controls (100%), we have found different body mass increments at the age of 37 days, e.g. 102.85%, 109.52% and 120.38% respectively for the three Luezea groups; 101.9% for Biostrong; 104.95% for Ecovit, and 103.47% for the group with the addition of the whole Luezea seeds. Thus, we can clearly see the largest increase of the body mass with 5% addition of Luezea seeds. Statistical analysis revealed that differences between the controls and Biostrong, Ecovit or Luezea 0.2% were not significant at day 30 as well as at day 37 (P > 0.05). On the other hand, differences between the control group and 1% or 5% Luezea were statistically highly significant (P < 0.01 for Luezea 1% at day 30; P < 0.001 for Luezea 5% at day 30 as well as for Luezea 1% and 5% at day 37). We can conclude, therefore, that the dietary supply of this ecdysteroid-containing material may be more efficient or more convenient biostimulator for birds in comparison to the commercially available preparations tested.

In the group receiving 5% addition of Luezea we have observed apparent tranquillization of the behaviour and, what was more important, the roosters in puberty showed remarkably decreased incidence of spontaneous dermal defects. The results in Fig. 1, which clearly reveal anabolic effects of Luezea in birds, fully agree with the earlier literature data, reporting strong anabolic effects of Luezea in the mammals (Veraskouski et al., 1974; Syrov & Kurmukov, 1977; Kudzina et al., 1980; Syrov, 1984). As far as we know, the present findings in Japanese quails represent the first experimental evidence for anabolic action of Luezea in birds.

Recently Kotsyuruba et al., 1992, 1993) investigated some biochemical changes induced by 20-hydroxyecdysone on purine metabolism and vitamin requirements in chicken. They concluded that this compound would affect both the nongenome early phase of action (0–1 hr) as well as the late genome phase (24–72 hr after application). It has been also determined in rat that an intravenously applied, labeled 20-hydroxyecdysone might be rapidly eliminated from the blood stream (t 1/2 = 8 min), suggesting that there are
mechanisms preventing the accumulation of ecdysteroids in the blood (Mironova et al., 1982; Dzukharova et al., 1984). In this study we have also investigated the problem whether the quails, feeding on the diet containing 20-hydroxyecdysone, would show the presence of this compound in the blood. And, as a matter of fact, Fig. 2 documents clearly that the quails feeding on the diet containing ecdysteroids have indeed the correspondingly increased blood concentrations of these hormones. Without any doubt, the amount of 20-hydroxyecdysone circulating in the blood of the quails is directly proportional to the amount of ecdysteroid that has been ingested with food. Quite unexpectedly we have found the highest amounts of 20-hydroxyecdysone immunoreactive equivalents in the group where the quails had a chance to consume deliberately the whole seeds of *Leuzea*. We have not included this experimental group into statistical comparisons with the other groups, since the uncontrolled consumption of the seeds of *Leuzea* would invariably affect final composition of the ingested food. We believe that such altered composition of the feed mixture may be responsible for relatively small anabolic effects found in this group (see Fig. 1), although the blood concentrations of ecdysteroids have reached very high levels in this group (80 ng per ml of blood serum). These concentrations are close to the levels of 20-hydroxyecdysone in insect haemolymph. The group of quails that received the Biostrong preparation had also slightly increased ecdysteroid levels in the blood. This might be due to the presence of certain ecdysteroid-containing plant ingredients.

The fact, that ecdysteroid concentration circulating in the blood of the quails has been directly proportional to the amount of dietary ecdysteroid suggests that metabolic breakdown and inactivation of these compounds in the circulatory system of birds cannot be so fast in comparison to that reported for the mammals. Finally, we would like to point out, that a major problem in all these studies with the whole plant or with plant extracts depends on the possibility of obtaining side effects due to other biologically active secondary plant substances. It is known, for example, that *Leuzea* contains all kinds of other important, and potentially also biologically active, organic compounds (for more details see Verskouski et al., 1974; Szabó et al., 1984; Szendrei et al., 1984; Varga et al., 1986; Girault et al., 1988). This problem can be solved only by comparison of these results with a new series of experiments performed with pure 20-hydroxy-ecdysone. These experiments have been already initiated. Among some important factors in evaluation of the nutritive value of food in poultry breedings we can find efficiency of food utilization and its conversion.
into the body mass. According to our data, the best food conversion has been found in the group receiving the polyfactorial probiotic composition Ecovit. On the other hand, the quails receiving 1% and 5% addition of *Leucaea* revealed accelerated onset of egg laying (for 4 days) and, in addition, there was complete elimination of the initial nonstandard eggs.*

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**References**


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* The first large scale feeding experiment with pure 20-hydroxyecdysone in Japanese quails has been terminated in October 1994. The concentrations equivalent to 1% *Leucaea* and 5% *Leucaea* seed exhibited similar anabolic effects as described in this paper, confirming thus that 20-hydroxyecdysone has been indeed responsible for the anabolic action of *Leucaea* seeds in Japanese quails.