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Use of Implants Regarding Growth Parameters in Black Iguana Young Maintained in Intensive Conditions

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Abstract

Implants are used in animal production to increase weight-gain. Due to this, the objective of this study was to evaluate one implant's effect on the black Iguana's (*Ctenosaura pectinata*) weight gain parameters in intensive conditions. Twenty-four iguanas' young were used in the study, at the age of 13 months old. The experiment lasted nine months. The subjects were kept in individual cages. Two types of commercial feed products were used combined with the use of the implant. The evaluated variables were daily weight gain (mg anim^{-1} d^{-1}) and longitudinal growth (µ anim^{-1} d^{-1}): total, snout-vent and of the head. The in vivo apparent digestibility of dry matter and feed conversion (mg) was also measured. In order to analyse these variables, a completely randomised block design, with 2x2 factorial arrangement and six repetitions, was adopted. The initial weight was used as a covariable. The sample results show that daily weight-gain and longitudinal growth ($p < 0.06$) were different depending on whether their food type was turkey feed with the implant, or chicken feed without the implant. The study concludes that turkey feed combined with the implant Zeranol increased daily weight-gain and total growth of the black iguana young in captivity.

Keywords: Feed; Zeranol; Metabolizable Energy; Crude Protein

Abbreviations

UMA: Units for Wildlife Conservation, Management and Sustainable Exploitation; Turkey feed (TFT); CP: Crude Protein; CF: Crude Fibre; CF: Crude Fat; CFT: Chicken Feed.

Introduction

The Units for Wildlife Conservation, Management and Sustainable Exploitation (UMA) allow the propagation of species and the elaboration of products and sub-products that can be incorporated into legal wildlife trade [1]. Captive upbringing systems have been developed for alternative species such as the endemic black iguana *Ctenosaura pectinate* [2], which is catalogued as a prioritised, endangered species in Mexico [3,4]. Due to this, it was important to include the *Ctenosaura* species in the Appendix II of the International Trade of Endagered Wild Fauna and Flora Species so as to regulate trade and ensure that it does not directly contribute to the extinction of the species, and to detect and combat illegal traffic of similar species [5]. Nevertheless, this species is used for human consumption, rural medicine, religion, jewellery, decoration and as pets [6-9].

While at the UMA, research and sustainable exploitation are permitted [10]. Its cultural uses lend ecological and economic importance to the species, and thus, research and extractive use of the animal are not permitted in the free life departs or derivatives of the black iguana [10]. Therefore, it is necessary to implement new alternative methods to improve the efficiency of productive systems in captivity [11]. One technique applied elsewhere is the use of anabolic implants that promote the iguanas´ growth. Implants have already shown their effectiveness in domestic animals in terms of cost-effective and efficient weight-gain [12]. Growth-promoting implants are used to fatten animals in confinement. Implants can promote growth more efficiently, and improve carcass muscle performance, without affecting quality characteristics. To obtain the maximum benefits from the implants, the nutritional diet must be adequate, in order to maximize the response of the implanted animals, since the availability of high-quality diets must be complementary to the use of implants to improve the growth response [13]. Free-roam iguanas take two to three years to reach adulthood [14]. However, with the use of implants they could become reach adult size in less time. We opted for an anabolic that has been shown to be effective in increasing the weight of animals in several countries [15]. Knowing that the use of implants in captive black iguana young could contribute to the species conservation, enable the repopulation of the species, preserve genetic diversity and provide alternative modes of rural economic development [16]. This present study poses the hypothesis that the Zeranol implant can improve growth parameters. The objective was to evaluate the effect of Zeranol on productivity in the black iguana *C. pectinata* in intensive conditions.

Material and Methods

Area of Study

This research was conducted at the Iguana Reproduction and Conservation Center of the Universidad del Mar (CECOREI-UMAR). Key code: INE/CITES/DGVS-CR-IN-0668-OAX./00 and NRA: CCRSY2006711. Located in Bajos de Chila, Mixtepec, Oaxaca, at Kilometer 128.1 of Federal Highway No. 200, Pinotepa-Puerto Escondido, Oax., Mexico. The coordinates of the location are 15°55′33.1′′ N and 97°09′02′′ W, with an elevation of 12 m above sea level [17].

The climate is warm and sub-humid. Relative humidity averages 79.1% and average annual rainfall of 987.6 mm, mostly concentrated in summer. The rainy season is considered to last from May to October. The average temperatures range between 22.4 and 32.6°C. The highest average temperature in the coolest month is 26.0°C [18].

Animals and Duration of the Experiment

Twenty-four iguanas (*C. pectinata*) were used in the study, at the age of 13 months old. They were not categorized by sex due to the complexity in differentiating sexes at this age and because research has not indicated any interaction between sex and feeding as factors in research regarding the growth of iguanas at this age [16,19]. The average initial weight was 71.3 ± 15.3 g, the average total length was 43.9 ± 4.8 cm, snout–vent length was 13.0 ± 0.9 cm and head length was 3.2 ± 0.2 cm. The experiment lasted nine months.

The subjects were kept in individual cages measuring 45 cm long, 45 cm wide and 45 cm deep [20]. The cages were made of wood; the walls and ceiling were covered with a mesh screen, and the central section of the roof was covered with wood to provide shade and allow the young to regulate body temperature by intermittent exposure to sun and shade. These conditions were prepared to protect the animals from rain, wind or excess light [21,22]. Cage cleaning was performed every day throughout the experiment.

Feeding

All animals were offered water and fed everyday ad libitum at 8:00 am. The iguanas were fed with two commercial feed types: 1) Turkey feed (TFT) with 12% humidity (H), 28% crude protein (CP), 5% crude fibre (CF), 2% crude fat (CF), 10% ash, 1.3% Ca and 0.9% P. And 2) Chicken feed (CFT) with 12% H, 21% CP, 9% CF, 2% CF, 10% ash, 0.9% Ca and 0.5% P. Nutrient information was obtained from product labels.

Treatments

Four treatments were used, each with a different combination of food and implant use: 1) TFT with an implant. 2) TFT without an implant. 3) CFT with an implant. 4) CFT without an implant. In the beginning of the experiment, the iguanas were dewormed and the implant was also applied according to the corresponding treatment. The implant had 4 mg of Zeranol (Bago-Pell®, BIOGÉNESIS-BAGÓ) applied subcutaneously in the iguanas' left dorsal. Those without the implant only received a puncture disturbance The Federal Law of Animal Health [23].

Evaluated Variables

The variables recorded were daily weight gain, DWG (with a OHAUS brand 700 series, 2.610 g capacity, 0.01 g approximation). DWG was measured using the following equation: DWG (mg anim-1 d-1) = (final weight g – initial weight g /270)*1000. Three length measurements (mm anim-1 d-1) were taken using methodology from Ortiz GJJ, et al. [21]: 1) total length from tip of the mouth to the tip of the tail (TL). 2) snout-vent length from the tip of the mouth to the vent (SVL), and 3) head length (HL) (using metric measurement tape and a caliper with 0.1 and 0.01 approximation respectively). Growth was calculated using the following equation: growth (µ anim-1 d-1) = (final length cm – initial length cm/270)*1000. The dry matter intake (DMI, mg anim-1 d-1) was calculated using the following formula: DMI = (dry feed intake/270)*1000 [14]. This amount was included as a percentage of the iguanas´ live weight. To determine the in vivo apparent digestibility of dry matter, four faeces samples were collected in the third week of each month for treatment. Previously samples from all months were prepared together to analyse dry matter [24]. Food conversion was determined as the ratio of milligrams feed intake/milligram gain [25]. The content of crude energy in each of the feeds used was calculated using the Merrill AL, et al. [26] methodology, and the percentage of metabolizable energy for iguanas (%ME) was calculated using methods from Van Merken LWD [27].

Statistical Analysis

The results were analysed as a completely randomised block design with 2 x 2 factorial arrangement and six replications. One factor was the type of food, and another was whether or not the implant had been applied. Furthermore, initial weight was considered as a covariable [28]. For this study the value α = 0.06 was used to detect any differences.

Results

The different feed types implant, and interaction between the feed and implant had no significant effect (p > 0.06) on daily weight gain, SVL and HL in young iguanas with the Zeranol implant. However, the turkey feed generated a greater (p < 0.06) daily total length gain compared to iguanas fed with chicken feed. After adjusting for the initial weight covariable, the feed treatment with concentrate for turkeys combined with the implant showed differences (p < 0.06) in daily weight gain and longitudinal growth compared to the chicken feed without implant treatment (Table 1).

Effect	Daily weight gain (mg)	Daily length growth (µ)		
		Total	Snout-vent	Head
Main Factors				
Feed	NS	p=0.06 $(F_{1,20})$=3.95	NS	NS
Implant	NS	NS	NS	NS
Interaction Feed*Implant	NS	NS	NS	NS
Turkey Feed Effect		43.4[a]		
Chicken Feed Effect		33.8[b]		
Treatments with Initial Weight Covariable				
Turkey Feed with Implant	487.4[a]	451.6[a]	209.8	22.8
Turkey Feed without Implant	380.6[ab]	404.1[ab]	191.3	23.7
Chicken Feed with Implant	384.4[ab]	347.1[ab]	178.8	22.8
Chicken Feed without Implant	338.7[b]	341.07[b]	175.8	21.2
Means	397.8	386	188.9	23.4
Standard error of the means	29.53	2.13	0.73	1.39

[a,b] Different superscripts in the same column indicate difference (p < 0.06).

Table 1: Effect of the application of Zeranol on average daily growth in 13- to 22-month-old black iguanas *C. pectinata*.

The dry matter intake as a live weight percentage, digestibility of dry matter and feed conversion contributed by the feed were not affected (p > 0.06) by factors of feed, implants, interaction between feed and implant, nor by the combination of treatments adjusted for a covariable. Metabolizable energy was affected (p > 0.06) by factors of feed and treatments adjusted for a covariable (Table 2).

Effect	DMI[†] (mg)	DMI[†] (%)	DDM[‡] (%)	FC[§]	ME[¶] Mcal kg^{-1}
Main Factors					
Feed	NS	NS	NS	NS	p<0.0001 $(F_{1,20})$=4.9E7
Implant	NS	NS	NS	NS	NS
Interaction Feed*Implant	NS	NS	NS	NS	NS
Turkey Feed Effect	NS	NS	NS	NS	2.644[b]
Chicken Feed Effect	NS	NS	NS	NS	2.762[a]
Treatments with Initial Weight Covariable					
Turkey Feed with Implant	1193.8	0.871	73.4	2.6	2.644[b]
Turkey Feed without Implant	1111.6	0.941	73.4	3.2	2.644[b]
Chicken Feed with Implant	1038.4	0.878	77.33	3.1	2.763[a]
Chicken Feed without Implant	932.8	0.803	78.47	2.9	2.763[a]
Means	1069.14	0.873	75.95	2.9	2.718
Standard Error of the Means	54.66	0.04	1.63	0.2	0.068

[†]DMI: Dry matter intake d^{-1}. [‡]DDM: Digestibility of dry matter. [§]FC: Feed conversion. [¶]ME: Metabilizable energy.
[a,b] Different superscripts in the same column indicate difference (p < 0.06).

Table 2: Effect of the application of Zeranol on consumption, digestibility and food conversion on average daily growth in 13- to 22-month-old black iguanas *C. pectinata*.

The different feed type had effect (p < 0.06) on total length. There was no effect (p > 0.06) on the final weight and snout-vent and head length achieved at 22 months of age due to the type of feed, implant or interaction in *Ctenosaura pectinata* (Table 3). Despite this, considering the initial weight covariable, total longitudinal growth and final weight was greater (p > 0.06) where turkey feed was used in conjunction with the implant compared to chicken feed without any implant.

Effect	Weight (g)	Length (mm)		
		Total	Snout-vent	Head
Main Mactors $(F_{1,20})$				
Feed	NS	0.06	NS	NS
Implant	NS	NS	NS	NS
Interaction Feed*Implant	NS	NS	NS	NS
Turkey Feed Effect	NS	56.58[a]	NS	NS
Chicken Feed Effect	NS	52.04[b]	NS	NS
Treatments with Initial Weight Covariable				
Turkey Feed with Implant	203.27[a]	58.33[a]	18.67[a]	3.92
Turkey Feed without Implant	174.43[b]	54.82[ab]	18.25[ab]	3.82
Chicken Feed with Implant	174.13[b]	51.17[b]	17.83[ab]	3.77
Chicken Feed without Implant	163.12[b]	52.92[b]	17.67[b]	3.72
Means	178.74	54.3	18.1	3.8
Standard Error of the Means	9.63	1.2	0.28	0.06
Probability	p = 0.054	p = 0.042	p = 0.06	p > 0.06

[a,b] Different superscripts in the same column indicate difference (p < 0.06).

Table 3: Effect of the application of Zeranol on weight and length gain in 13- to 22-month-old black iguanas *C. pectinata*.

Discussion

The greater total weight gain and longitudinal growth in young *Ctenosaura pectinata* was due to the higher content of crude protein (31.8%) in dry poult feed compared to the 23.9% content in chick feed, which instead contains more amino acids. This coincides with the report from Ortíz GJJ, et al. [21] who found that black iguanas fed with 21.2% dry crude protein and increasing levels of lysine increased their growth and weight gain, without exceeding a maximum level of growth. They concluded that Lysine was possibly the first limiting amino acid for this species.

Regarding the *C. pectinata* which were given turkey feed in combination with the implant, at 22 months old, total longitudinal growth was 1.2% less, but 25% greater than the average daily weight gain compared to results reported by Arcos-García JL, et al. [14]. The difference in weight gain is explained by the fact that the latter used 16 to 22% crude protein and 13.7 to 25.5% neutral detergent fibre with tomato-based feed (*Lycopersicon esculentum*), alfalfa (*Medicago sativa*), fly larvae (*Notonecta unifascinata*) and commercial rabbit feed. Thus, even if cecal generated energy were high, the limiting factor regarding growth could be due to diet and metabolism in the cecum.

Another study on reptiles indicate that the application of implants has no effect on size and weight gain, for example, in juvenile specimens of swamp crocodile *Crocodylus moreletii* [29].

It has previously been reported in free-roam adult iguanas that the feed of the cecal content is from 14.5 to 25.3% CP, with 31.6% insoluble neutral detergent fibre [30,31]. Due to this, it is expected that the young iguanas' protein requirements be higher than this value. The latter is likely the reason for which there was an increase in weight gain and longitudinal growth in this study using turkey feed. The CP content in feed consumed by iguanas varied depending on their age: 1) for hatchlings, insects form the main part of their diet. 2) for older juvenile iguanas, insects form part of the diet, but the consumption of leaves is more prominent. 3) for adult iguanas, leaves are the most important part of the diet and insects are rarely consumed [32].

Dry matter intake increased by 150% compared to intake at six months of age [33], and 99% for young iguanas at nine months of age. This is considered normal due to the greater size of the iguana in this study and is in keeping with the fact that the greater the animal size, the more they consume [25].

The particle size of the turkey and chicken feeds were very similar in this experiment. The inconvenient factor of different grain size and feed volume were avoided [34]. It has been mentioned that the main limiting factor of poultry feed for iguanas can be the high content of starch, low level of neutral detergent fibre, and additives included in the manufactured feed [34]. This is detrimental to bacterial activity which degrades fibre in the iguana´s cecum Vélez-Hernández L, et al. [30] and which can contribute nutrients and energy necessary for growth.

Corresponding to previous studies on smaller iguanas, feed consumption as a percentage of live weight fluctuates between 1.49 and 1.63 [19,33]. In this study, consumption (%) was lower, which could indicate that at a greater age, dry matter intake as a percentage decreases. Perhaps due to ectothermic animal's lesser need for energy and because they require a smaller quantity of nutrients for their growth and development [22]. In this experiment, metabolizable energy in the chicken feed measured 2.763 Mcal kg^{-1}, and 2.644 Mcal kg^{-1} for turkey feed. This is considered normal compared with 2.193 to 2.74 Mcal from metabilizable energy kg^{-1} feed [7,30] Therefore, energy supplied is not considered to be limited.

Feed conversion registered for this study presents similar values to those (2.3 to 3.9) in black iguana between two and six months old [33]. However, they are different from those indicated by Rueda-Zozaya P, et al. [19], who reported a feed conversion range between 4.5 and 6.5. This is due to the higher amount of neutral detergent fibre in the diets used in the latter study which suggest that iguanas need a higher content of FDN in their diets to aid cecal activity.

Dry matter digestibility is similar to the reported 73.5 to 74.6% in two to six-month-old black iguanas consuming diets made up primarily of insects and plants [33]. On the other hand, digestibility in diets based on chicken and rabbit feed had a dry matter digestibility of 41.5 and 59.9%, respectively due to its neutral detergent fibre content [19].

Taking the iguanas' final weight into account, there is a similarity with the predicted weight which postulated that at 22 months of age, the iguanas would weigh 170.91 g (with a range between 137.2 to 204.8 g) [35].

These values appear similar to recorded figures in this study and confirm that the growth prediction for the species is adequate. Taking this into account, the iguanas which were fed with turkey feed in combination with the implant, although achieving higher weight gain, were still within the normal limits of growth for black iguanas.

According to Zubieta RTL [31], iguanas grow nine times slower than chickens and need the same amount of food to develop. In other words, iguanas need 24 months to gain two kg in live weight, the optimum weight for human consumption. These characteristics make the prospect of rearing captive black iguanas unappealing.

It is important to consider genetics in experiments with *Ctenosaura pectinata*, given that it is a wild species presenting high genetic variability. It is recommended to choose genetic groups (a block, or repetitions by maternal or paternal origin) a technique used in previous studies which reduces experimental error and encourages a greater possibility of the treatments showing more reliable results [20,21]. By reducing genetic variability, future treatments may reveal differences in response variables.

Another factor to consider is the reptiles´ internal temperature given that ectothermic animals require an external source of heat [22]. Regarding this study, one of the limiting factors in terms of adequate metabolism was the rainy season, due to which the outdoor temperature was reduced by rainfall, wind speed and the presence of clouds. This caused the reptiles to modify their feed consumption, and affected their growth.

Vélez-Hernandez L, et al. [30] recorded a concentration of cellulolytic bacteria which oscillate between 9.2 x 10 and 3.5 x 10 g-1 of cecal content. This is similar to domestic species and indicates that the bacteria in *C. pectinata* are active at around 37°C. In this regard, in the study of Allison CA, et al. [36] in which 41 caged young *Cyclura nubila* were kept at room temperature, the average rectal temperature of this species was found to be similar to the temperature of its environment. Likewise, it has been indicated that *Ctenosaura pectinata* must also maintain their body temperature at around 35.4°C in order to obtain optimum live weight gain and food consumption [34].

The justification for not taking sex into account in this study was that on finishing the experiment, the sexual differentiation of the iguanas male:female was 1:1, which suggests that all of the treatment types had the same probability of positive or negative expression in response variables relating to sexual differentiation.

The difference in growth between males and females is visible starting around 18.3 months of age [16]. Results corroborate until the age of nine months [19] where it has been indicated that food consumption and growth are similar between males and females.

In relation to the lack of results on the use of implants, the following considerations must be taken into account. In homeothermic animals, implants are reapplied every ninety days behind the ear [37,38]. In the case of the iguanas, the implants used were administered for a period of nine months considering that they have a slower metabolism [22]. Therefore, the implant placement site was adequate, because the release of the active substance could be even slower due to the ectoderm's physiology.

In addition, the reptilian metabolic rate is less than one third to one tenth compared to mammals of the same size, the black iguana being quite efficient in incorporating energy from the diet for growth in the breeding stage [39].

Possibly the most important aspect for the iguana's metabolism to work properly with the implant is related to body temperature and energy consumption. When taking into account the energy balance point; if the energy released by the iguana into the environment is greater than the energy consumed, then it will cool down and use the metabolic energy for its vital maintenance processes (basal metabolism) and possibly lose fat and weight [40]. Due to this, it is important to carry out further studies in which temperature is strictly controlled, whereby the iguanas can reach a rectal temperature above 35.4°C.

Conclusion

In sum, this study concludes that in the young black iguana *Ctenosaura pectinata* ranging from 13 to 22 months of age, weight gain improves with feed with a high crude protein content (31.8% in dry matter) and the use of the implant Zeranol. The metabolizable energy concentration of 2.644 kg-1 of feed was that which stimulated the greatest weight gain of the juvenile black iguana *C. pectinata*.

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Conflict of Interest Statement

The authors declare that there are no conflicts of interest.

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