Implementation of soybean (Glycine max) as a fed complement for *Caiman latirostris* with commercial purposes.

Introduction. Sustainable use is a strategy to conserve crocodilian species and their habitats, often linking local communities and livelihoods to economic incentives for conservation (Ross, 1995). The ranching program for broad-snouted caiman (*Caiman latirostris*) in Santa Fe Province, Argentina is a good example of this strategy, where economic incentives have been generated for local rural communities involved with the program (Larriera and Imhof, 2006). Through this State-regulated program, *C. latirostris* eggs are collected from wild habitats and artificially incubated, with subsequent rearing of hatchlings in farms.

Captive-reared caimans are typically fed commercially available, extruded, floating pellets made with animal protein. Hatchlings are usually fed a 50–56% protein diet (dry weight basis) for the first 4–6 months, followed by a 45–50% protein diet until harvest at approximately 10–12 months of age. Regardless of the species, feed costs tend to be one of the most significant expenses of crocodilian farming (Rodríguez et al., 1996; Pinheiro and Lavorenti, 2001), therefore research efforts have often aimed at reducing feed costs (Isberg, 2007). The use of less expensive sources of protein, from plants is evaluated as a more economical and ecologically friendly alternative to animal protein (Reigh and Williams, 2013, 2014, 2016, 2018).

Despite crocodilians being considered obligate carnivores, many surveys of free-ranging populations across multiple age classes and in multiple species have recovered plant matter from their gastrointestinal tracts (Magnusson et al., 1987; Thorbjarnarson, 1993; Brito et al., 2002; Wallace and Leslie, 2008; Platt et al., 2013). Alligatorids species appears to have the ability to digest and assimilate crude protein and carbohydrates from a variety of plant sources, including corn, grain, soybean meal, soy protein isolate, and wheat gluten (Staton et al., 1990a; Staton et al., 1990b; Reigh and Williams, 2013, 2018; Tracy et al., 2015; Hilevski and Siroski, 2020).

Based on the hypothesis that crocodilians could ingest plant material to gain nutritional benefits, the objectives of this study were to: determine the digestibility of plant nutrients by *C. latirostris* and evaluate the optimal amount of soybean to include in their food.

Methods

Animals and housing. Broad-snouted caimans used in this study were hatched from wild, artificially incubated eggs, and captive-reared at "Yacarés Santafesinos" facilities (Gobierno de Santa Fe/MUPCN). Caimans were selected from four different nests to avoid "clutch effect" (Bagatto et al., 2012). The animals were housed in plastic tanks, each 0.6 m high with a total area of ~1 m2 [divided into land (33%) and water (67%; 6-10 cm deep) areas]. Air and water temperatures were maintained at 31 ± 1°C, and tanks were cleaned and refilled with warm water (27-30°C) daily before animals were fed.

Diets and feeding schedules. A) Digestibility trials. Twenty-four caimans were used. They were initially purged with 1.5 mL of Lactulon® per day, for two days (Hilevski and Siroski, 2020) and then forced-fed daily with a corresponding experimental diet (Table 1) through a nasopharyngeal probe into the stomach (Ayarzaguena, 1983) for 7 consecutive days. **B) Growth trials.** Forty-eight caimans were fed ad libitum three times per week with their corresponding experimental diet (Table 2) for 90 consecutive days. The three experimental diets were fed to caimans at a rate of 20% of BM per day. (Table 1).

Table 1. Composition of experimental diets to digestibility trials. 0SM: 0% of Soybean meal; 20SM: 20% of Soybean meal; 40SM: 40% of Soybean meal; 60SM: 60% of Soybean meal. Chromic oxide is an indigestible marker.

Component	0SM	20SM	40SM	60SM
Crude protein from egg albumin (%)	99.9	79.9	59.9	39.9
Crude protein from soybean meal (%)	0	20	40	60
Indigestible marker (Cr_2O_3)	0.1	0.1	0.1	0.1

Table 2. Composition of experimental diets to growth trials. 0SM: 0% of Soybean meal; 25SM: 25% of Soybean meal; 40SM: 40% of Soybean meal.

Component	0SM	25SM	40SM
Soybean (%)	0	25	40
Chicken head (%)	100	75	60

Analytical methods. Twenty-four hours following the last force-feeding, animals were sacrificed according to protocols approved by the Ethics and Safety Advisory Committee of the Faculty of Veterinary Sciences of the National University of the Litoral, Santa Fe, Argentina (resolution "FCV 0966480-19"). At that time, stomachs and intestines were collected. Stomachs were examined for digesta residues, the large intestine was separated from the small intestine and the latter was divided into three equal segments (Tracy et al., 2015). All digesta residues were removed from each intestine segment, weighed and frozen immediately. Digestibility of each treatment diet was established through the methods of Brisson (1956), Furukawa and Tsukahara (1966). Digestibility of soybean meal was determined with a standard equation (NRC, 1983).

Data of food consumption (FC) of each experimental diet was recorded. We knew initial dry weight of food provided, twelve hours after feeding the animals the food remains were remove from the tank and put to dry in an oven during twenty-four hours at 65 °C. Twenty-four hours following the last feeding the animals were weighted and measured from the snout to the tails tip (total length - TL) every thirty days from the beginning of the trial until day 90. The food conversion rate (FCR) was calculated for every animals of each experimental diet by using dry weight of food consumed and body mass gain (BMG) for individuals in any experimental diet.

To determine the optimal value of SM inclusion in the food to promote the major growth of captive *C. latirostris*, we used the mean values of BMG, and total length gain (TLG) obtained through the growth trials. These values allowed us to generate parabolic equations which were used to calculate the maximum values of BMG and TLG according to the percentage of soybean inclusion in the food.

Statistical Analysis. The distribution of the data was determined by assessment of histograms, the Shapiro–Wilk test and the Levene's test. Kruskal-Wallis and Mann-Whitney tests were used to detect differences in digestibility of total diet and soybean meal. One-Way ANOVA was applied to identify differences in FC and FCR of the diet between the treatments. Two-Ways Repeated Measures ANOVA and Tukey tests were used to detect variations in BMG and TLG between the experimental diets and through the time. These analyses were performed with commercial software (SPSS version 22, IBM Corporation, Armonk, New York 10504, USA), and statistical significance was set at alpha 0.05.

Results. The digestibility of soybean meal (Table 3) varied according to the diet ($K = 8$, $P = 0.018$, $n = 18$). Total digestibility of soybean meal was calculated using the digestibility values of each small intestine segment and averaging them. 20SM was slightly lower than diets 40SM and

60SM (U = 3, P = 0.0202, n = 18). Digestibility in DSI differed between the levels of soybean meal (K = 8.222, P = 0.0163, n = 17), with the digestibility of diet 60SM higher than 20SM (U = 2, P = 0.0224, n = 17), and 40SM (U = 4, P = 0.0306, n = 17).

Digestibility of soybean meal in 60SM between segments of intestine did not show differences. However, digestibility of 20SM was higher in PSI and MSI than DSI (U = 3, P = 0.0357, n = 20), and LI (U = 0, P = 0.0281, n = 20). Similar results were observed in 40SM, where the digestibility was higher in PSI than MSI (U = 5, P = 0.0453, n = 21), DSI (U = 0, P = 0.0050, n = 21), and LI (U = 0, P = 0.0281, n = 21). Likewise, digestibility of MSI was greater than DSI (U = 2, P = 0.0130, n = 21).

Table 3. Digestibility coefficients of soybean meal in each segment of intestine. PSI: Proximal Small Intestine; MSI: Middle Small Intestine: DSI: Distal Small Intestine; LI: Large Intestine.

Diet	Segment of intestine				Total
	PSI	MSI	DSI	LI	
20SM	94.296	90.821	84.373	28.348	89.941
40SM	98.731	96.683	90.907	74.659	95.441
60SM	94.832	95.635	95.602	87.114	95.523

Total digestibility of diets was calculated using the digestibility values of each small intestine segment and averaging them. Values of digestibility were different between them (K = 17.3, P = 0.0006, n = 24) and were between 97 and 99%. 0SM was slightly lower than 20SM, 40SM, and 60SM (U = 0, P = 0.0050, n = 24) (Table 4). The same case occurs with the digestibility of diets in different segments of the intestine. In PSI digestibility of diets was different (K = 15.07, P = 0.0017, n = 24), 0SM was lower than 20SM (U = 0, P = 0.0082, n = 24), 40SM (U = 1, P = 0.0050, n = 24), and 60SM (U = 0, P = 0.0081, n = 24). Digestibility in MSI was also different according to the diet (K = 11.85, P = 0.0079, n = 22). Digestibility of 0SM was lower than 20SM (U = 4, P = 0.0306, n = 22), 40SM (U = 0, P = 0.0050, n = 22), and 60SM (U = 0, P = 0.0142, n = 22). In DSI digestibility was different between diets too (K = 16.05, P = 0.0011, n = 22), 0SM was lower than 20SM (U = 3, P = 0.0357, n = 22), 40SM (U = 0, P = 0.0050, n = 22), and 60SM (U = 0, P = 0.0050, n = 22). Furthermore, 60SM was higher than 20SM (U = , P = 0.0224 , n = 22), and 40SM (U = , P = 0.0202, n = 22). On the other hand, in the LI, digestibility was not different between diets.

Digestibility of treatment 60SM between segments of intestine does not have a significant difference. However, digestibility of the control diet was lower in PSI than MSI (U = 1, P = 0.0032, n = 23), DSI (U = 0, P = 0.0050, n = 23) and LI (U = 0, P = 0.0081, n = 23). The same results were found with the digestibility of 20SM, it was lower in PSI than MSI (U = 4, P = 0.0306, n = 22), DSI (U = 0, P = 0.0050, n = 22) and LI (U = 0, P = 0.0142, n = 22). Something similar happened in 40SM, in which digestibility was lower in PSI than DSI (U = 0, P = 0.0050, n = 23), and LI (U = 0, P = 0.0050, n = 23).

Table 4. Digestibility coefficients of diets in each segment of intestine.

Diet	Segment of intestine				Total
	PSI	MSI	DSI	LI	
0SM	97.658	97.750	95.827	53.113	97.079
20SM	99.774	99.413	98.377	59.630	99.221
40SM	99.965	99.796	99.965	78.884	99.575
60SM	99.788	99.809	99.718	93.311	99.777

Figure 1. Body mass of broad-snouted caiman fed with diet enriched with soybean meal in each time.

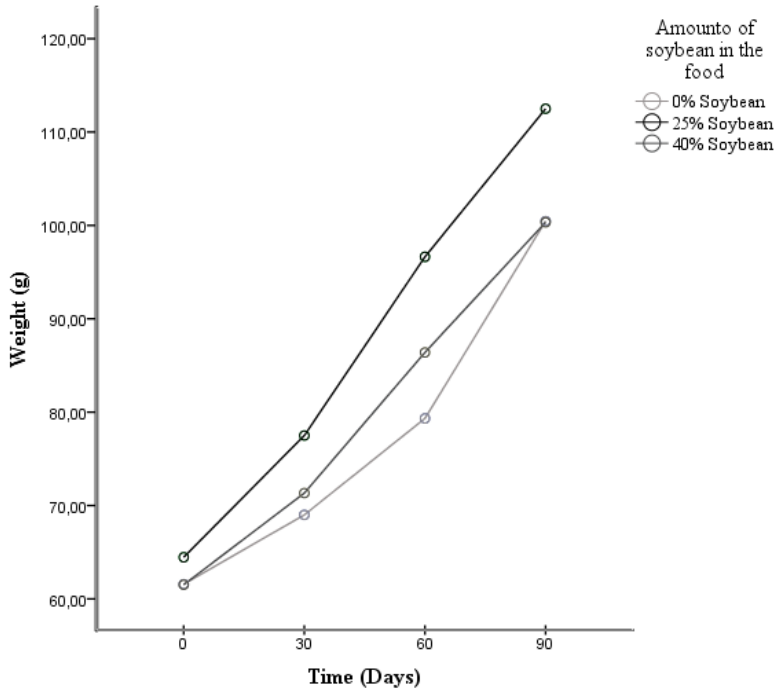
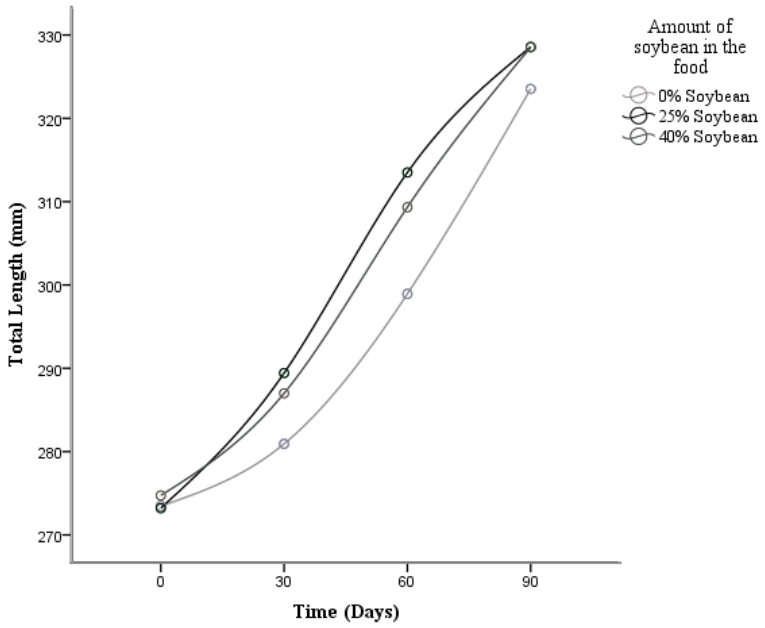


Figure 2. Total length of broad-snouted caiman fed with diet enriched with soybean meal in each time.



Caimans treated with their respective experimental diet had a significative raise in BM and TL through the experimental time [$P < 0.001$ (Figures 1 and 2)]. It can be seen in the Figures 1 and 2 that BM and TL were equal between the animals treated with the different experimental diets. However, the Figure 1 shows a trend where the BMG of animals from the experimental diet 25SM was higher than the others experimental diets. Figure 2 shows the same trend, the TL

of the animals treated with the experimental diets 25SM and 40SM was slightly higher than 0SM diet.

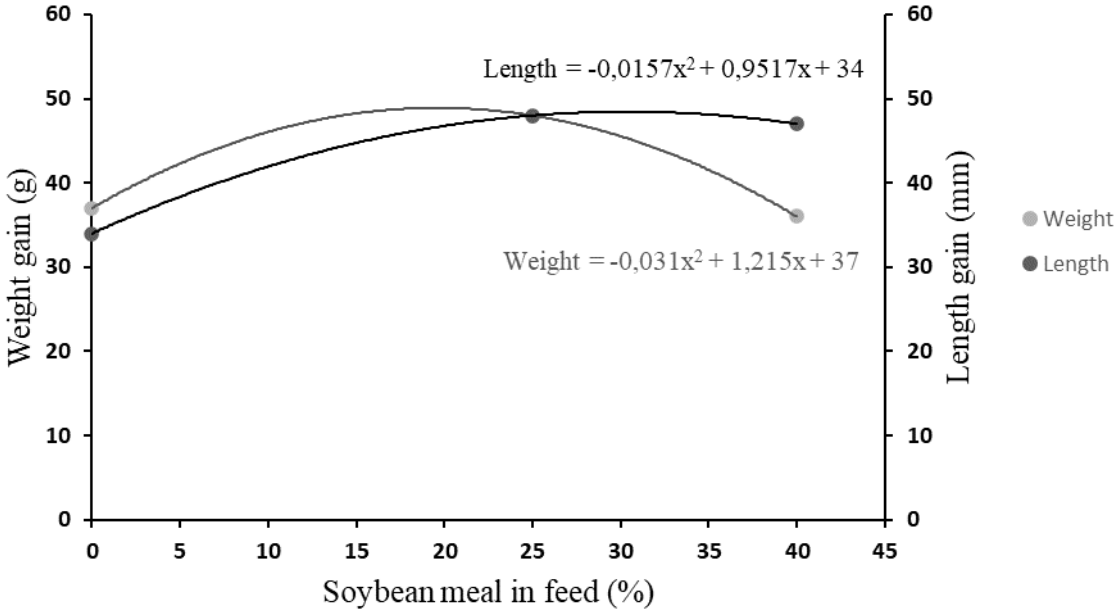
Food consumption was equal to 37.86 g ± 10.93 (SE) and were equal between the experimental diets (Table 5). Food consumption rate was equal to 4.41 g ± 0.66 (SE). Food consumption rate of the 25SM and 40SM diet were equal between them, but more efficient than 0SM ($P = 0.026$, $n = 38$; $P < 0.042$, $n = 38$) (Table 5).

Table 5. Mean values of food consumption (FC) and food conversion rate (FCR) by broad-snouted caiman fed with diets enriched with soybean meal.

Experimental Diet	FC (g/day) ± (SE)	FCR (g) ± (SE)
0SM	34.98 ± 2.08	6.95 ± 1.45
25SM	38.49 ± 2.97	2.89 ± 0.71
40SM	40.11 ± 3.85	3.27 ± 0.74

Figure 3 shows the mean of BMG and TLG at three months of growth trials and 0, 25, and 40% of SM inclusion in the food. We can see the parabolic equations which were used to calculate the values of BMG and TLG according to the percentage of soybean inclusion. Both curves intersect at 25% of SM inclusion in the food, which suggests that it is the optimal value of SM inclusion in the diet, matching with the performance of this experimental diet (Figure 1 and 2; Table 5).

Figure 3. Optimal inclusion of soybean meal in food to broad-snouted caiman.



Conclusion. In summary, results of this study indicate that broad-snouted caiman efficiently digested soybean meal and efficiently growth with diets based on this ingredient, reaching their optimal growth when this is provided at levels of 25% of the diet, because at more than 60% could oversaturating the digestion capacity of the stomach. Our results indicate broad-snouted caiman should have a digestive system adapted to take advantage of nutrients from vegetables like soybean without interfering with their health. It is important to note that there may be differences between broad-snouted caimans consuming whole soybeans in the wild and the laboratory trials, where they are feeding on "modified" plant material, with a broken cell wall

and allowing access to the proteins, carbohydrates, and lipids. This information is useful to feed manufacturers developing practical crocodilian diets. It is important to emphasize there are differences between alligatorids and crocodiles with respect to their ability to digest plant nutrients; thus it is not possible to extrapolate results to all crocodilians species. Additional research is necessary to determine optimum nutrient levels and ingredient combinations for farm-raised crocodilians fed compounded diets.

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