Research Article

Diet preference and breeding success in captive-bred Greater rheas (*Rhea americana*): a preliminary study

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Abstract: We conducted 2 consecutive experiments to verify whether adult captive Greater rheas (*Rhea americana*) could select diets differing in nutritional and energetic content, and to evaluate the effect of those diets on egg production and hatchability. From August to January, animals were offered four diets: processed feed for rheas (diet 1), diet 1 + soybean (diet 2), processed feed for chicken (diet 3), and diet 3 + soybean (diet 4). Rheas preferred the diets containing feed for chicken (diets 3 and 4, 179,67 ± 12.12 [S.E.] g/ind./day). Diet 2 was more consumed (95.67 ± 16.70 g/ind./day) than diet 1 (60.09 ± 11.31 g/ind./day), whereas preferences for feed for chicken did not vary with supplementation. Diet 1 and soybean-supplemented diets were consumed by males (113 ± 13.66 and 163.57 ± 13.33 g/ind./day, respectively) in a greater amount than by females (42.27 ± 8.61 and 96.72 ± 16.52 g/ind./day, respectively). Diet type did not affect egg production (43-63 eggs), but hatchability was 13% higher in eggs from females fed soybean-supplemented feed (diets 2 and 4), and those fed diet 2 produced more chicks. While diets 3 and 4 were apparently of lower nutrient quality, they provided more energy than those formulated with processed feed for rheas. Conversely, diet 2 seemed to be the most favourable for females during the reproductive season. The quality of diets provided to females would largely influence breeding success in captive Greater rheas.

Key words: Greater rheas, breeding success, diet preference

Introduction

The Greater rhea (*Rhea americana*) is the largest flightless bird in South America, reaching 93-140 cm in height and 22-28 kg in weight (1). In the 2000s, captive breeding of rheas has become an agricultural venture and a strategy for ex situ conservation (2). However, no sound feeding guidelines that help to optimise captive breeding of this native ratite have been provided to date.

Egg chemical composition is one of the keys to normal embryo development and successful hatching. The egg yolk fatty acids provide 90% of the energy required for embryo development (3), and their proportion highly depends on the diet. Soybean (*Glycine max*) has widespread use in poultry feeding because of its high nutritional value, suggesting that it might also be a worthy dietary supplement for rheas. This legume has highly proteinaceous oil-rich seeds, with a high content of linoleic and linolenic acids (4), which cannot be synthesised and therefore must be provided directly by the feed (5). A high linolenic acid concentration in the egg yolk of some birds including ratites has been specifically associated with better reproductive performance, such as increased egg hatchability (2,3) and chick survival rate (2,6). However, similar yolk fatty acid content was found in

eggs from captive Greater rhea females fed on soybean-supplemented diets and from females occurring in the wild (7).

Different species, including birds, are capable of selecting their food based on its quality and on individuals' nutritional requirements; thus, animals adjust their nutrient intake to their metabolic requirements (8), which are greatest at the reproductive stage (9). In Greater rheas, both sexes have critical nutritional requirements, since females produce a large number of eggs in each breeding season (average in captivity: 40 eggs of 617 g each) (10) and males perform incubation and chick caring, allocating nearly 22% of their body weight to these activities (11). Female rheas have the ability to reserve nutrients before reproduction (12), suggesting that the quality of diets consumed during the pre-reproductive period may influence breeding success. Thus, one would expect rheas to select foodstuffs, achieving the balanced and optimum nutrients' intake. Despite behaving as selective herbivores in the wild (2), captive rheas accept a wide range of feedstuffs. Taking advantage of this characteristic, producers usually employ feedstuffs that are not formulated to meet the specific requirements of this ratite to reduce feed costs (12). Most producers opt for combining pastures (*Medicago sativa*, *Cichorium intybus*, etc.) with processed feeds for chicken, instead of providing comparatively expensive processed feeds for rheas. Moreover, dietary nutrients for rheas are often based on extrapolations of those from other ratites.

The objectives of this study were to determine if adult Greater rheas bred in captivity select feedstuffs rich in nutritional and energetic content and to evaluate the a posteriori effect of diets offered on egg production and hatchability.

Materials and methods

Two consecutive experiments were conducted on an experimental Greater rhea farm located at the Estación Experimental Agropecuaria San Luis (Instituto Nacional de Tecnología Agropecuaria, INTA) in Villa Mercedes (Argentina). The first experiment explored feed selection and the second evaluated egg production and hatchability responses to the diets offered. In both experiments, the following diets were offered: 1) Aliba® processed feed for breeder Greater rhea (diet 1), 2) diet 1 supplemented with soybean (diet 2), 3) Vasquetto® processed feed for finisher chicken (diet 3), and 4) diet 3 supplemented with soybean (diet 4). The processed feeds selected were pelleted and of brands widely used in the region. However, they differed mainly in nutrient content and colour (possibly associated with their components). The processed feed for Greater rhea was green, whereas that for chicken was yellow. Moreover, the latter contained less crude protein, crude fat, and crude fibre, but greater energy (Table 1) than the former. Soybean was added at the expense of daily processed feed ration for Greater rhea (7) and chicken (13), by half. Soybean nutrient and energy values were obtained from results reported by Giorda and Baigorri (4). The resulting rations, based on consumption of 600 g per day, contained 1380, 1928, 1560, and 2064 Kcal for diets 1 to 4, respectively. Although soybean has antinutritional factors (lectin and trypsin inhibitors), it poses no risk to birds when appropriate chemical or heating inactivation is performed (4). In all the seeds used in this work, heat-processing was conducted through vapour, and the absence of trypsin inhibitor activity was confirmed using the method of Liu and Markakis (14) at the Instituto de Ciencia y Tecnología de los Alimentos (ICTA) of the Universidad Nacional de Córdoba, Argentina.

Experiment 1

Feeding preference was evaluated in August, a pre-reproductive month for Greater rheas in Argentina. The breeding season of this species extends from spring (September) through mid-summer (January/February), both in wild and captive conditions (15). Sixteen adult Greater rheas were distributed in two 20 × 30 m pens: 5 males (2 years old) in one pen and 11 females (3 and 5 years old) in the other. Five males per corral was considered the maximum acceptable number in the pen to avoid conflicts and fight among them. Four big feeders were placed in each pen and 600 g/animal/day of each diet was offered through each container. This amount was higher than that of the consumption curve for Lesser rhea (16), to avoid feed depletion. Consumption of each diet was recorded twice daily (between 0900 and 1030 and between 1800 and 1930) by weighing feed

Table 1. Chemical composition of processed feed provided to Greater rheas in the experiments evaluating food preference, egg production, and hatching success.

Content	Processed feed for Greater rhea (breeder)	Processed feed for chicken (finisher)
Protein (%)	20	16.8
Metabolisable energy[B] (kcal/kg)	2300	2600
Maximum crude fibre (%)	14	6
Lipids (%)	7.1[A]	4.4
Linolenic acid (%)[A] of total fatty acid composition	3.9	3.6
Linoleic acid (%)[A] of total fatty acid composition	38.9	40.3
Lysine (%)	0.96	0.8
Methionine (%)	0.35	n/a
Methionine + cystine (%)	0.65	0.52
Vitamin A (IU/kg)	17500	0
Vitamin D3 (IU/kg)	4750	3300
Vitamin E (IU/kg)	200	40
Vitamin K3 (mg/kg)	10	3
Vitamin B1 (mg/kg)	6	2
Vitamin B2 (mg/kg)	18	8
Vitamin B6 (mg/kg)	5	3
Vitamin B12 (mg/kg)	0.07	0.025
Pantothenic acid (mg/kg)	35	n/a
Nicotinic acid (mg/kg)	60	45
Folic acid (mg/kg)	6	1.5
Calcium (%)	2.5	0.90-1.10
Phosphorus available (%)	0.45	0.48-0.68
Chromium (mg/kg)	12	n/a
Manganese (mg/kg)	150	75
Zinc (mg/kg)	160	55
Iron (mg/kg)	45	27
Iodine	n/a	1
Selenium	n/a	0.25

Available data were provided by manufacturers
[A] From Lábaque (11)
[B] Calculated based on tabular values reported by Rostagno et al. (26)

offered and refused. After a 7-day acclimation period, data were collected for 7 days. Greater rheas were maintained under similar conditions during these 2 periods. Because there were no differences in feed consumption between morning and afternoon feeding in both males and females (males H = 2.14, P > 0.05; females H = 2.30, P > 0.05), all data were pooled to obtain a daily consumption value. Feeding preference was evaluated with a 3-way ANOVA using sex, type of processed feed, and soybean supplementation as factors with 2 levels each (males vs. females, processed feed for rhea vs. chicken, and soybean-supplemented vs. non-supplemented processed feed, respectively). As consumption data

were recorded daily, days were previously incorporated into the ANOVA model as blocks, to control for between-day dependence. Nevertheless, this block effect was non-significant ($F_{6.6}$ = 0.593, P = 0.73), and so it was finally excluded from the analysis. Post-hoc comparisons between factor levels were made with Tukey's HSD tests.

Experiment 2

To evaluate egg production and hatchability in response to the diet consumed, another experiment was conducted later, using 8 females and 4 males of those employed in Experiment 1. Females were selected based on their known-breeder status and similarity of origin, age, and body size to avoid possible confounded reproductive performance (12). Sample size was limited by availability of adult individuals of similar characteristics, pen size, and tolerance of individuals to conspecifics. Approximately 30 days before the start of laying, individuals were distributed in four 20 × 30 m pens; 2 females and 1 male were assigned to each pen, where they received one of the 4 experimental diets. Feeds were offered in the same amount in all pens. This 30-day period was used because birds generally store fatty acids during ovarian maturation (17) and especially because formation of reproductive structures of Greater rhea females would start 1 month before the onset of laying (11).

Eggs were collected daily, marked with a pencil for pen identification, and taken to 2 automatic turning forced-air incubators/hatchers with a capacity of 45 rhea eggs each, as described by Navarro et al. (18). Hatching rate did not differ between incubators (19). During the incubation period, embryo development was periodically monitored using an ovoscope, and eggs that were infertile, contaminated, or with dead embryos were discarded. To determine the influence of the diets on the number of eggs laid and egg hatchability, a χ^2 goodness-of-fit test and a χ^2 test of independence were used, respectively. As Greater rhea is an undomesticated species of large body size and difficult to manipulate, it was not possible to replicate treatments. However, in captivity each female can lay about 40 eggs throughout the breeding season (15), ensuring the availability of a high number of eggs per female for the experiment.

Results

Feeding preference

Considering all diets together, feed consumption was affected by sex ($F_{1.48}$ = 8.944, P = 0.004) and type of processed feed used ($F_{1.48}$ = 104.828, P < 0.001), but not by soybean supplementation ($F_{1.48}$ = 0.075, P = 0.784). Rhea males consumed on average more feed (532.3 g ± 20.92 [S.E.]) than females (455.64 g ± 36.95), and both sexes showed differential preference for processed feeds (sex × processed feed: $F_{1.48}$ = 17.424, P < 0.001). This difference was due to the higher amount of one type of processed feed consumed by males (feed for rheas in diets 1 and 2) than by females (P < 0.001). However, the other type of processed feed (for chickens, comprising diets 3 and 4) was strongly preferred over the feed for rheas (males: P < 0.001; females: P < 0.001) and consumed in similar quantities by the individuals of both sexes (P = 0.83) (Figure 1a).

The further analysis including soybean supplementation revealed important interactions between the 2 factors considered (sex and type of processed feed). Males and females showed different preferences for soybean supplementation (sex × soybean supplementation: $F_{1.48}$ = 13.942, P < 0.001): while males preferred the soybean-supplemented diets (2 and 4) over the non-supplemented ones (P = 0.03), females showed the opposite trend (P = 0.082). Although both sexes consumed similar quantities of feeds corresponding to non-supplemented diets (P = 0.95), males consumed more soybean-supplemented feeds than females (P < 0.001) (Figure 1b).

Soybean supplementation also affected the preference for different processed feeds (processed feed × soybean supplementation: $F_{1.48}$ = 10.913, P = 0.002). The birds showed a trend to consume a larger quantity of soybean-supplemented processed feed for rheas, compared to the same processed feed without soybean (P = 0.068), whereas the consumption of processed feed for chicken was the same, regardless of soybean supplementation (P = 0.155) (Figure 1c).

Egg productivity and hatchability

Egg laying started on 21 September and ended on 17 January. During this period, a total of 215 eggs were collected from the 4 pens. Egg laying did not start simultaneously, the earliest females being those
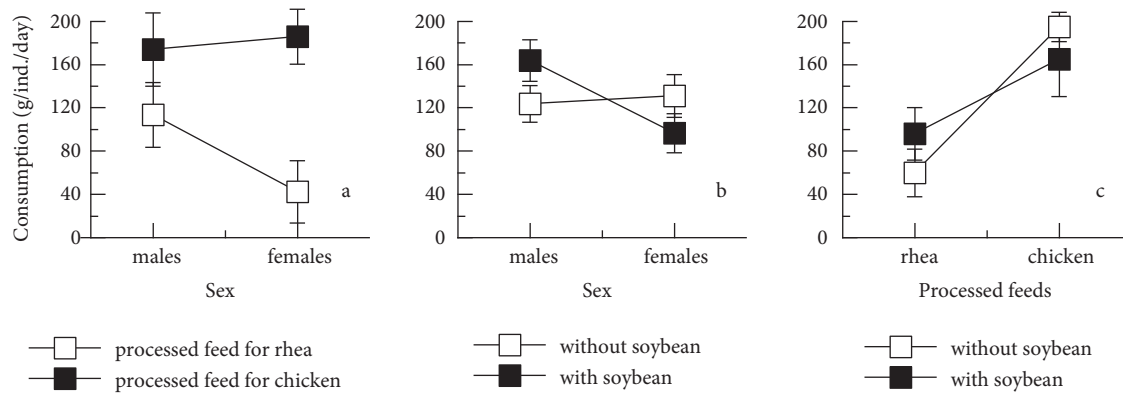
Figure 1. Average consumption [± S.D.] of different diets by captive rheas as a function of sex and type of processed feed (a), sex and soybean supplementation (b), and type of processed feed and soybean supplementation (c).

fed on diet 3 (21 September), followed by those on diet 4 (13 October), those on diet 1 (27 October), and lastly those on diet 2 (6 November).

There was no effect of diet on egg production (χ^2 = 3.98, d.f. = 3, P > 0.05) or hatchability (χ^2 = 4.78, d.f. = 3, P > 0.05). As this result might be due to the relatively small sample size, we decided to pool data by group to evaluate if egg production and hatchability were greater in females fed on diets with soybean supplementation (diets 2 and 4) or without it (diets 1 and 3), using a goodness of fit test and a one-tailed Fisher's exact test, respectively. Although the inclusion of soybean in the diet did not improve egg production (χ^2 = 0.88, d.f. = 1, P > 0.05), hatchability was greater in eggs laid by females that consumed soybean supplement (Fisher's exact test, P < 0.05) (55%) than in eggs laid by females only consuming processed feed (43%). As a result, the females that consumed soybean supplement produced more chicks (either in absolute quantity—as a measure of fitness—or relative to the number of eggs laid—as a measure of cost/benefit) than females that only consumed processed feed (Table 2).

Discussion

Greater rheas selected the diets composed of processed feed for chicken over those of processed feed for rhea, this preference being particularly more marked in females. A greater consumption of a high-energy diet by males (diet 4) during the pre-reproductive stage would allow them to undergo longer fasting periods. As males are normally in charge of the complete incubation of the clutch, by selecting this type of food they would increase their chances of successfully producing chicks, posing a

Table 2. Effect of diet on number of eggs laid, hatchability, and number of chicks produced.

Type of diet	No. of eggs laid	% hatched	No. of chicks produced
Processed feed for rheas	43	51	22
Processed feed for rheas + soybean	52	60	31
Processed feed for chicken	63	36	23
Processed feed for chicken + soybean	57	51	29

lower threat to their own survival. This behaviour would be consistent with the theory of optimal foraging, which postulates that individuals would consume those foods that provide more energy and maximise their fitness (20).

Female preference for processed feed for chicken resulted in an earlier onset of laying and enhanced egg production. However, among the diets offered, those supplemented with soybean (diets 2 and 4) improved hatchability by 13% with respect to non-supplemented diets. Indeed, the highest hatchability value, and therefore the highest number of chicks, was recorded in the group fed diet 2. Hatching success under the latter diet reached the mean value mentioned by Navarro and Martella (15) for captive Greater rhea (60%) and was 9.6% higher than that observed on this experimental farm during the previous breeding season (P.E. Vignolo, pers. com.). Thus, diet 2 (processed feed for rheas supplemented with soybean) is apparently, among the diets offered, the one of greatest quality and the most favourable for females during the breeding season. This fact may be due to the higher content of linolenic acid in the processed feed for rheas (3.9% of total fat acid composition) (12) and in soybean (11.7%) (7), than in processed feed for chicken (3.6%) (12). Such direct relationship between the content of linolenic acid in the diet and hatchability has been already suggested for rheas (2), ostriches (3,6), and other birds (5).

Since hatchability of eggs from females fed on processed feed for chicken is comparatively low with respect to that from females fed on processed feed for rheas, the question that needs to be answered is why females preferred the former, as this selection would be reducing their fitness. Other factors not related to diet quality, such as sensory attributes of food (taste, texture, colour, etc.) that sometimes play a key role in nutrient intake (21), may have influenced their selection. Fibre content may have also affected selection by females, which preferred to consume a greater amount of the feed with the lowest crude fibre content (processed feed for chicken, Table 1). Thus, processed feed for chicken is probably more palatable for females and, even though it is not the food of greatest energy content, it may provide an adequate amount of energy for egg production. However, this food may lack other important nutrients for normal embryo development (e.g., essential fatty acids, proteins, vitamins), and this is further reflected in a lower hatchability. Accordingly, Brand et al. (22) and Cooper et al. (23) indicated that energy content in the diet of African ostriches is the main limiting factor for egg production. However, the amounts of proteins, minerals, amino acids, and vitamins that ostrich females receive before the formation of the first egg are important to achieve a good reproductive performance (23).

Additionally, the length of the period when females are provided with the feed of highest quality, before the onset of egg laying, might also influence females' breeding success (24). Thus, Greater rhea females may require more than the 30-day period monitored in this work to accumulate sufficient nutrient reserves and consequently exhibit significant changes in reproductive parameters. Our results suggest that, as in other birds (23-25) diet quality would have a great effect on the breeding success of captive Greater rheas. Hence, to obtain an adequate cost-effectiveness relationship, it is necessary to define the suitable combination of energy and nutrients in the diet of Greater rhea during the reproductive season, as well as the length of the period needed to supplement females' diet before egg laying starts.

We found that, despite being less preferred, processed feed for rheas showed the highest hatchability, suggesting that the quality of the diet provided to the adult females would largely influence breeding success. Although preliminary, the results obtained here could be useful not only for Greater rheas but also for other ratite species in captivity.

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