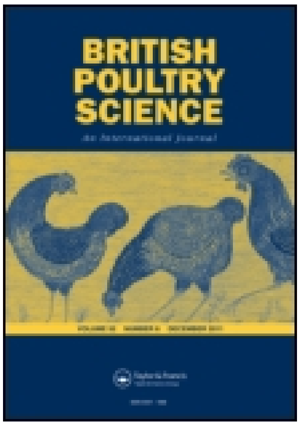

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The influence of diet composition on egg and chick traits in captive Greater Rhea females

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Abstract 1. This study was conducted to evaluate the influence of diet composition on egg number, physical and chemical characteristics of eggs and weight and survival of chicks throughout a breeding season in a captive-bred population of greater rheas (*Rhea americana*).
2. From August to December, individuals were offered two diets: processed feed for rheas and processed feed for chicken (which is the feed most commonly offered to farmed rheas in Argentina). Reproductive performance of 15 females was monitored and female body weight was recorded before egg-laying onset. Within each experimental group, the following variables were determined: egg morphometric variables and percentage of components, fatty acid composition, hatching success and initial weight of chicks and mortality during the first week of life.
3. Females that were fed on processed feed for rheas delayed onset of laying and reduced laying period and number of eggs produced. However, females of this group laid larger eggs, with higher percentages of yolk and yolk lipids, and exhibited higher hatching success and chick weight compared with those that received chicken diet. Survivorship of chicks in their first week of life was not affected by composition of the diet offered to parental female.
4. Some reproductive parameters of captive greater rhea females fed on processed feed for rheas were higher than those of individuals receiving processed feed for chicken.

INTRODUCTION

The diet supplied to any captive-bred species generally plays a significant role in most performance traits (Klasing, 1998; Speake *et al.*, 1999; Aganga *et al.*, 2003; Cooper *et al.*, 2005; Bazzano *et al.*, 2011). The feed consumed by captive ostriches (Noble *et al.*, 1996) and greater rheas (Navarro *et al.*, 2001) is quite different in nutrient composition from food consumed in the wild (Martella *et al.*, 1996; Ullrey and Allen, 1996; Klasing, 1998; Aganga *et al.*, 2003). Accordingly, captive birds produce eggs with a very different fatty acid profile from those of their counterparts living under natural conditions (Noble *et al.*, 1996; Speake *et al.*, 1999; Surai *et al.*, 2001*a*). In ratites, the yolk of eggs from captive-reared ostriches (*Struthio camelus*) and greater Rheas (*Rhea americana*) has a lower proportion of linolenic fatty acid than that from their wild counterparts (Noble *et al.*, 1996; Navarro *et al.*, 2001; Lábaque *et al.*, 2010).

Most nutritional studies in ratites have been conducted in ostriches (reviewed by Angel, 1996; Ullrey and Allen, 1996; Aganga *et al.*, 2003; Cooper *et al.*, 2005), because it was the first and is currently the most commonly farmed ratite species (Gillespie and Schupp, 1998; Cloete *et al.*, 2004; Ipek and Sahan, 2004; Lambrechts, 2004; Fair *et al.*, 2005; Cloete *et al.*, 2006). Thereafter, when rhea farming started to grow as an agricultural venture, the need for information about nutrient requirements was met by direct extrapolation from that available for

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ostriches (Angel, 1996; Carbajo Garcia *et al.*, 1997; Aganga *et al.*, 2003). Consequently, scientific information on specific nutrition demands of greater rheas is still very scarce (Dominino *et al.*, 2006; Bazzano *et al.*, 2011). Bazzano *et al.* (2011) reported that most rhea producers in Argentina provide their stocks with pastures (*Medicago sativa*, *Cichorium intybus*, etc.) and processed feed for chicken, even when a processed feed for rheas (Aliba®) is available in the market. The latter is not widely used because it is more expensive to produce than chicken processed food, and as it is produced only in one site in the country, distribution costs increase their retail price in distant places. Surprisingly, although processed feed for chicken lacks important nutrients for embryo development in ratites, greater rheas seem to select it against the processed feed for rhea (Bazzano *et al.*, 2011). As it was suggested in broilers (Yo *et al.*, 1997), this preference may be related to some sensory characteristics of the feed, which have been found to play a key role in nutrient intake in poultry species.

Lábaque *et al.* (2010) suggested that greater rhea stocks should be provided with a good-quality diet, particularly well before the start of the breeding season, because weight gain before the onset of egg laying will increase bird performance and egg and chick quality. According to these authors, greater rhea females that attain a threshold body weight at the beginning of the laying season might improve their nutritional status, which is associated with good health condition and a great availability of reserves for the production of larger eggs and/or of a greater number of eggs (Lábaque *et al.*, 2010). Thus, the amount and quality of nutrients accumulated by females before laying onset could determine their reproductive performance in that season. Here we evaluate the incidence of two diets (processed feed for chicken and for rheas) on reproductive performance of greater rhea females taking into account their apparent preference for processed feeds for chickens (Bazzano *et al.*, 2011). A study was conducted to evaluate the influence of diet composition on the number and physical and chemical characteristics of eggs and initial weight and first-week mortality of chicks throughout a breeding season in a captive-bred population of greater rheas (*R. americana*).

MATERIALS AND METHODS

Experimental population

During the period from 2002 to 2003, 19 adult greater rheas of the population maintained in an experimental field at the Zoo of Córdoba (Argentina) were divided into two groups (1 male: 3.5 females) in which three age categories (hatched 2, 3 and ≥4 seasons before) were equally represented. The birds were individually identified with neck tags and/or numbered plastic leg bands and kept under similar conditions in paddocks of about 700 m^2 each, fenced with 1.6-m high rhombic wire mesh. Since hatching, all animals were given balanced feed formulated for chicken. In mid-August (one month before the average date of start of the laying season), each female was weighed using a 90 × 60 cm platform digital scale (0.1 kg accuracy). After that date, the individuals of one group were fed on a daily 600-g ration composed of balanced feed formulated for rheas (Aliba®), whereas the other group was provided with the same amount of balanced feed formulated for chicken. The latter group was considered as the control group because it was fed on the most frequently used diet in commercial rhea farms in Argentina.

Diet composition

Diet composition was analysed by collecting samples from 8 feed bags to provide the range of variation between different feed bags during the experiment. Nitrogen content was determined (AOAC, 1980) and protein values were estimated on a conversion factor of 6.25 times total nitrogen. Lipids were extracted from 10 g of dry matter using *n*-hexane in a Soxhlet apparatus for 10 h. Lipid content was quantified by weight difference (AOAC, 1980). Fatty acid analysis was conducted following the same procedure described for eggs (see section Performance variables).

From the analysis performed, it was observed that lipid and protein content and percentage of palmitic, palmitoleic, oleic and linolenic fatty acids were greater, and stearic, linoleic and saturated fatty acid were lower in balanced feed formulated for rheas (Aliba®) than in that formulated for chicken (control group) (Table 1). In addition, Bazzano *et al.* (2011) mentioned that feed formulated for chicken contained a lower amount of crude fibre (6% vs. 14%), but greater energy (10.9 vs. 9.6 MJ/kg) than the Aliba® feed. The other chemical characteristics mentioned in Table 1 were provided by the manufacturer.

Performance variables

Throughout the laying season (September to December), rheas were monitored daily by direct observation (between 11.00 h and 19.00 h) to record the number of eggs laid and identify the laying female that laid the egg. All the eggs (54 from the control group and 33 from the Aliba® group, respectively) were collected immediately after being laid and individually marked with pencil; egg fresh weight was measured to the nearest

Table 1. *Chemical composition of the rations fed to two captive groups of Greater Rheas*

Content	Processed feed for Greater Rhea (breeder)	Processed feed for chicken (finisher)
Protein (%)	20	16.8
Metabolisable energy[B] (MJ/kg)	9.6	10.9
Maximum crude fibre (%)	14	6
Lipids (%)	7.1	4.4
Fatty acids:		
16:1 (palmitoleic)	0.9 ± 0.4[a]	1.3 ± 0.3[b]
18:1 (oleic)	26.6 ± 0.3[a]	28.3 ± 1.1[b]
18:2 (linoleic)	40 ± .7.5[a]	38.9 ± 1.7[b]
18:3 (linolenic)	3.6 ± 0.8[a]	3.9 ± 0.5[b]
Total saturated (SFA)	22.3 ± 6.1[a]	20.0 ± 2.1[b]
Total unsaturated (PUFA)	71.3 ± 6.1[a]	72.3 ± 2.1[b]
PUFA/SFA	3.19	3.61
Vitamin E (IU/kg)[A]	200	40
Vitamin K3 (mg/kg)[A]	10	3
Vitamin B1 (mg/kg)[A]	6	2
Vitamin B2 (mg/kg)[A]	18	8
Vitamin B6 (mg/kg)[A]	5	3
Vitamin B12 (mg/kg)[A]	0.07	0.025
Pantothenic acid (mg/kg)[A]	35	n/a
Nicotinic acid (mg/kg)[A]	60	45
Folic acid (mg/kg)[A]	6	1.5
Calcium (%)[A]	2.5	0.90–1.10
Phosphorus available (%)[A]	0.45	0.48–0.68
Chromium (mg/kg)[A]	12	n/a
Manganese (mg/kg)[A]	150	75
Zinc (mg/kg)[A]	160	55
Iron (mg/kg)[A]	45	27
Iodine[A]	n/a	1
Selenium[A]	n/a	0.25

[A]Available data were provided by manufacturers; [B]Calculated based on tabular values reported by Rostagno *et al.* (2000).
Mean values within the same row sharing a common superscript letter are not statistically different at $P < 0.05$ (Mann–Whitney test).

0.1 g and egg length (major axis) and breadth (minor axis) were measured with a calliper (to the nearest 0.1 mm). Their volume was estimated using the modified formula of Hoyt (1978): $L \times B^2 \times K_w$, where L = length (cm), B = breadth (cm) and the constant K_w = 0.578 (for greater rhea; Lábaque *et al.*, 2007). As 80% of females lay eggs every 2 to 4 d (Fernández and Reboreda, 1998; pers. obs.), to obtain a number of eggs per female that allows performing analyses of their physical and chemical characteristics, the eggs were stored at 13°C to 16°C for 48 h. This temperature range and storage period ensured that egg characteristics and potential hatchability remained unaltered (Lábaque *et al.*, 2004), which is essential for further hatching success.

Of the total eggs collected, 16 (11 from the control group and 7 from the Aliba® group) were used for physical and chemical analyses. One to two eggs per female were randomly selected to measure physical and chemical characteristics. These eggs were stored at 4°C until processed, following the procedure of Navarro *et al.* (2001). After storage, the eggs were weighed, their volume was determined gravimetrically and density was calculated. Later, each egg was broken out and the yolk, albumen and shell were weighed and separately processed. Each liquid component was dehydrated at 60°C to constant weight. Nitrogen content was determined (AOAC, 1980) and protein values were estimated as 6.25 times total nitrogen. Lipids were extracted from 10 g of dry matter using 2 chloroform:1 methanol (v/v) in a Soxhlet apparatus for 8 h. Lipid content was quantified by weight difference (AOAC, 1980). Fatty acid analyses were carried out from yolk lipids extracted with 2 chloroform:1 methanol (v/v) at room temperature. Lipids were subjected to alkaline saponification (1 N potassium hydroxide in methanol) and the unsaponifiable was matter extracted with *n*-hexane. The fatty acid methyl esters (FAMEs) were obtained using 1 N sulphuric acid in methanol and analysed by gas chromatography (GC) according to Lábaque *et al.* (2010). All chemical determinations were conducted in duplicate for each egg.

Incubation conditions

Of the total eggs collected, 43 from the control group and 26 from the Aliba® group were artificially incubated in multistage, 45-egg capacity, forced-air incubators, with automatic 3-h turning intervals, and at a humidity level that allowed eggs to lose 12% to 15% of their initial mass during the 38-d incubation period (Navarro *et al.*, 1998). All eggs were weighed and candled every 4 to 5 d thereafter. Eggs with no embryonic development were regarded as infertile, whereas those with arrested embryonic development were classified as embryonic mortality. In both cases, eggs were discarded and opened to confirm the absence of embryo development. Fertility was assessed by opening the egg in 89% of the cases, whereas in the remaining 11% it was determined by candling. Fertile hatchability was calculated as hatched eggs/fertile eggs.

At 34 d of incubation, eggs were transferred to a hatcher set at the same temperature and humidity as the incubators. The hatcher trays had individual compartments that allowed matching the chick with the egg from which it hatched. Twenty-four to 36 h after hatching, the chicks were weighed and marked with a numbered leg band made of coloured PVC plastic adhesive tape. The number of chicks surviving the first week of life was recorded.

Statistical analyses

Data were analysed using the statistical software INFOSTAT 1.0 (2001). Mean ± Standard Error values are presented throughout the text. Mann–Whitney tests (Zar, 1984) were used to compare the average mean body weight of females, clutch size and characteristics of eggs and chicks produced of both the groups because the data did not conform to the assumptions of the ANOVA. Chi-squared contingency tests were used to compare the fertile hatchability of eggs and chick survival (the first week of life) between the groups.

RESULTS

The Aliba® group started to lay eggs on 9 October and finished on 8 December, whereas the control group started on 30 September and finished on 15 December (length of the laying period: 47 and 61 d, respectively). No differences in body weight were observed before the start of the breeding season between females belonging to the control group (23.18 ± 0.88 kg, n = 5) and the Aliba® group (20.68 ± 0.73 kg, n = 5) (Mann–Whitney test, W = 35, P = 0.15). The average number of eggs laid per female in the Aliba® group (10.9 ± 1.1) was lower than that of the control group (12.4 ± 0.6) (Mann–Whitney test, W = 1173; P = 0.013). Morphometric variables and percentage of yolk were higher in the Aliba® group than in the control group. On the other hand, the latter group produced eggs with the lowest percentage of albumen (Table 2). The percentage of yolk lipids was higher but the total unsaturated fatty acid was lower in the Aliba® group than in the control group (Table 3). Fertile hatchability (100%) of eggs belonging to the Aliba® group was significantly higher than that of the control group (84%) (χ^2_{Yates} = 7.09, df = 1, P = 0.007). Regarding offspring traits, the initial weight of chicks (383.2 ± 5.7 g) was higher in Aliba® than in the control group (357.8 ± 6.2 g) (W = 1013.5, P = 0.005). However, chick survival in the Aliba® group (100%) did not differ from that of the control group (94.4%) (χ^2 = 1.49; df = 1, P = 0.22).

Table 2. *Physical characteristics of eggs laid by captive Greater Rheas fed on different diets*

Variable	Diet: Control[A]	Diet: Aliba®[B]
Weight (g)	555.7 ± 6.9[b]	589.3 ± 8.7[a]
Length (cm)	12.5 ± 0.1[b]	12.8 ± 0.1[a]
Diameter (cm)	8.8 ± 1.1[b]	9.0 ± 1.2[a]
Volume (cm^3)	562.1 ± 8.2[b]	602.6 ± 8.5[a]
Eggshell (%)	12.1 ± 0.4	12.7 ± 0.5
Albumen (%)	56.9 ± 1.4[a]	52.6 ± 0.9[b]
Yolk (%)	29.3 ± 1.3[b]	32.9 ± 0.9[a]

[A]Processed feed for chickens (finisher);
[B]Processed feed for Greater rhea (breeder).
Mean values within the same row sharing a common superscript letter are not statistically different at $P < 0.05$ (Mann–Whitney test).

Table 3. *Chemical composition of eggs laid by captive Greater Rheas fed on different diets*

Variable	Diet: Control[A]	Diet: Aliba®[B]
Yolk protein (%)	28.8 ± 0.7	27.2 ± 0.8
Yolk lipids (%)	58.4 ± 0.9[b]	59.4 ± 0.7[a]
Albumen protein (%)	75.6 ± 0.7	75.1 ± 0.8
Fatty acids:		
Saturated		
16:0 (palmitic)	25 ± 0.5	26.4 ± 0.9
18:0 (stearic)	7.7 ± 0.4	8.1 ± 0.4
Unsaturated		
16:1 (palmitoleic)	3.2 ± 0.2	2.8 ± 0.2
18:1 (oleic)	41.8 ± 1.1	39.7 ± 1.0
18:2 (linoleic)	15.3 ± 0.5	14.5 ± 0.4
18:3 (linolenic)	1.3 ± 0.1	1.4 ± 0.1
20:4 (arachidonic)	2.6 ± 0.2	2.7 ± 0.3
Total saturated	32.9 ± 0.8	35.0 ± 1.0
Total unsaturated	64.3 ± 0.6[a]	61.5 ± 1.1[b]
PUFA/SFA	0.59 ± 0.02	0.54 ± 0.02

[A]Processed feed for chickens (finisher);
[B]Processed feed for Greater rhea (breeder).
Mean values within the same row sharing a common superscript letter are not statistically different at $P < 0.05$ (Mann–Whitney test).

DISCUSSION

The slight delay of the date of laying onset (9 d) in the Aliba® group with respect to the control group might be attributed to the change in the food type made one month before the date of the laying onset reported in the literature (Lábaque *et al.*, 2003, 2004, 2010; Martella and Navarro, 2006). Hence, these individuals might have gone through a period of neophobia to the novel food (Marples and Kelly, 1999). In addition, greater rhea preference for balanced feed for chicken (Bazzano *et al.*, 2011) may have contributed to the occurrence of this behaviour.

In birds, the increase in egg size usually limits the number of eggs that an individual can produce (Brown, 1988; Heaney *et al.*, 1998; Lábaque *et al.*, 2010). Accordingly, the greater size of eggs produced by females of the Aliba® group than those of the control group might be a result of the average lower number of eggs deposited by the former group. In agreement with patterns observed in other bird species (Moss *et al.*, 1981; Williams, 1994; Deeming *et al.*, 1996; Carbajo Garcia *et al.*, 1997; Prinzinger *et al.*, 1997; Pelayo and Clark, 2003), eggs produced by females of the Aliba® group had a higher percentage of lipids, exhibited a 30% higher hatching success and produced larger chicks than females of the control group. The higher percentage of yolk and yolk lipids produced by the Aliba® group shows that there was a greater availability of nutrients to the developing embryo, which resulted in greater hatching success in that group. All these parameters would be related to a better quality of eggs and chicks produced in the group subjected to diet treatment. Indeed, these results are consistent with those reported by Lábaque *et al.* (2010) in terms of the influence of nutritional status of females, which depends on the quality/quantity of the diet received before the start of reproduction, on performance of the species at that stage. Therefore, the results of the present work confirm that the preference of greater rhea for processed feed for chicken reported by Bazzano *et al.* (2011) has negative effects on individual success; attempts should be made to replace this balanced feed with the one specifically formulated for greater rhea.

Furthermore, considering that in the greater rhea (Lábaque, 2006), as in other avian species (Sotherland and Rahn, 1987; Deeming *et al.*, 1996; Carbajo Garcia *et al.*, 1997; Prinzinger *et al.*, 1997; Pelayo and Clark, 2003), there is a high positive correlation between egg size and chick size, a higher initial weight of chicks hatched from females of the Aliba® group would be due to a greater size of eggs produced by that group. The fact that in precocial species, like the greater rhea, the greatest influence of physical and chemical characteristics of eggs on chick development would occur mainly in the earliest d of life (Anderson and Alisauskas, 2001; Pelayo and Clark, 2003) should be taken into account in the analysis of the relationship between female traits, diet offered and chick traits. Accordingly, in the present work, chick survival during the first week of life was very high (above 95%) and was not affected by the type of the diet offered to female parents. A possible explanation to this result is that intensive artificial breeding conditions to which chicks were subjected in the present work may have had a similar influence on chick development, regardless of parental traits and/or of the egg from where it hatched. Permanent availability of food and shelter might promote a favourable environment for growth of all individuals, masking potential effects of chick origin. Future experiments using rearing conditions of different welfare states might reveal different performances associated with individual origin.

In recent years, commercial rearing of greater rheas in Argentina seems to be going through a new stage characterised by strengthening of production (Navarro and Martella, 2008). Accordingly, the parameters of egg hatching and chick survival need to be optimised for a better development of this agribusiness. The better performance of reproductive parameters in the Aliba® group than in the control group shows the need for future studies evaluating the use of this feed under experimental conditions other than the ones used in the present work, with the aim of identifying the effects of this diet and designing management strategies to improve production in greater rhea farms. On the one hand, because processed feed for greater rhea is more expensive than chicken feed, the producer might feed the stock with the latter food type during part of the year and incorporate Aliba® some time before the start of the breeding season. This possibility requires evaluating when is the optimum time before the onset of the breeding season for incorporating the new feed so that greater rhea females can store enough nutritional reserves (Lábaque *et al.*, 2010). Indeed, avian females in general store fatty acids, which are essential for the functioning of reproductive structures, during ovarian maturity before egg laying (Noble and Cocchi, 1989). In the case of greater rhea females, development and formation of reproductive structures would start some 30 d before the onset of egg laying (Fernández and Reboreda, 1998). Hence, the specific feed should be incorporated at least 30 d before laying onset.

In ratites, such as the greater rhea and the ostrich, a greater proportion of linolenic acid has been recorded in wild eggs of both species than in eggs of farmed species, which is later reflected in a greater hatching success of eggs of wild environments (Noble *et al.*, 1996; Navarro *et al.*, 2001). In the present work, the profile of fatty acids in both experimental captive groups (Aliba® and control) was largely different from characteristic values of wild individuals, which is in agreement with differences observed in other avian species (Speake and Thompson, 1999; Speake *et al.*, 1999; Surai *et al.*, 2001*a, b, c, d*). The differences in egg composition found between wild and captive greater rheas should be taken into account in the reformulation of the composition of the feed offered to captive individuals with the aim of optimising the use of nutrients supplied under these conditions. Hence, according to the present findings and information on greater rhea provided in the literature (Navarro *et al.*, 2001; Lábaque, 2006; Lábaque *et al.*, 2010; Bazzano *et al.*, 2011), composition of balanced feed formulated for ratites might be modified to achieve increased percentage of linolenic acid, which is allocated to egg formation.

CONCLUSIONS

Consumption of feed specifically formulated for greater rheas improved the reproductive performance of the study population, although fewer eggs were produced. The incorporation of this feed type increased the size of eggs and, therefore, of chicks produced, as well as the percentage of yolk and yolk lipids in eggs laid and hatching success, without affecting mortality during the first week of life. This feed should be incorporated in the diet of breeders before the breeding season, but earlier than the one-month period used in the present work.

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