Propolis in the Diet of Blue-Fronted Amazons (Amazona aestiva spp): Digestibility of Minerals

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Research Article

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Abstract

The objective of the study was to evaluate the effect of propolis on the digestibility of minerals in the diet of blue-fronted amazon (*Amazona aestiva*). For this, 10 adult birds (5 males and 5 females) were separated into two groups (Control and Propolis 0.5% m/m). The apparent digestibility coefficient (ADC) of the following minerals was analyzed by spectrometry: calcium (Ca), phosphorus (P), iron (Fe), magnesium (Mg), sodium (Na), potassium (K), zinc (Zn), manganese (Mn) and nickel (Ni). ANOVA was used to evaluate ADC analyzes of the minerals, as well as the Pearson correlation between the ADC of the control and propolis rations. There were lower correlations in the ADC of the minerals of the birds that received propolis ration. It was concluded that parrots consuming propolis-containing ration showed better ADC levels, possibly due to the reduction in the interaction between the minerals and the alteration of the intestinal flora by propolis.

Introduction

The nutrition of Psittacidae went through several phases and the biggest challenge today is to formulate specific diets. The different needs in the life stages of the animal such as growth, maintenance, and production, make the formulation of diets a challenge to meet the individual demands in the pet market [1, 2].

As part of the diet, minerals - chemical elements that cannot be synthesized by living organisms and of vital importance in their survival, such as RNA and DNA composition for example - can be used to determine the appropriate nutritional balance. Mineral absorption may be affected by synergism or antagonism with each other and with vitamins, physical status, age, and intestinal villi, which is the site of absorption of most minerals [3, 4].

Various additives are used in animal nutrition to meet dietary requirements and improve the absorption of nutrients, especially minerals. Nowadays, propolis, a product produced by honeybees *Apis melífera* L., is composed of more than 300 substances (including minerals such as calcium, phosphorus, manganese, and zinc) and has several biological properties (immunomodulation, hepatoprotection, bactericide, and fungicide), have been used as an additive in chicken diets [6–10]. Researchers recommended propolis in broilers for reducing the negative effects of heat stress, indicating improved digestibility [11, 12]

Silva et al. [13] tested three levels of inclusion of propolis in commercial diets for blue-fronted amazons (*Amazona aestiva*), and the results showed an immunomodulatory and hepatoprotective effect at a dose of 0.5% propolis in the diet. However, there are no data on the effects of the inclusion of propolis on nutrition and digestibility for these birds. Therefore, the objective of this study was to evaluate the effect of propolis on the digestibility and correlation of minerals in the blue-fronted amazons' diet.

Material & Methods

Local of study

The experiment was conducted in the Wild Animals Sector of the Department of Animal Production, College of Veterinary Medicine and Animal Science, Universidade

Estadual Paulista (UNESP), Botucatu Campus, São Paulo State, Brazil. Approved by the ethics committee on the use of animals of this college, protocol number 191/2013-CEUA.

Animals and treatments

Ten blue-fronted amazons (*Amazona aestiva*) from the Center of Medicine and Research in Wild Animals (CEMPAS - UNESP - Botucatu), 5 males and 5 females (sexed, adult, and indefinite age) were distributed in individual cages (1, 40m x 0.60m x 0.50m) with free access to water and feed supplied in the amount of 40 grams daily at 7h a.m. All the birds participating in the experiment were pre-selected because they presented reduced or zero peeling behavior, reducing possible fecal contamination in the trays due to leftovers.

The experimental design used was a crossover, with two treatments and five replications, with each bird being one repetition. The project was carried out in two stages of the same duration (9 weeks), the first being carried out from February to April and the second from May to July 2015.

At the beginning of each stage, there was a two-week adaptation, in which all birds received commercial ration (PapagaioMix – Biotron®) without propolis addition. After two weeks, the experimental period started, with five birds in each treatment:

- Control Treatment (C): 0.0% of propolis in the total diet
- Propolis treatment (P): 0.5% of propolis in the total diet (0.20 g of propolis to 40 g of feed)

For the digestibility assay, the seventh week after the beginning of the treatment was used and the birds that participated in the control treatment in the first experimental period composed the propolis treatment in the second period and vice versa. Thus, the experiment was carried out in April in the first experimental period, and in July in the second, with a 12-week interval between them, ensuring that there was no residual effect on the birds that consumed propolis in the first stage.

Throughout the digestibility assay, 40 g of labeled feed (addition of chromium oxide) was supplied alternately to the water, avoiding the birds' natural behavior of wetting the feed before ingesting and the possibility of losing the marker by leaching. The experiment was carried out for 7 days and the collections of feces and leftover rations were carried out from the fourth to the seventh day.

We weigh the parrots three times: at the end of the adaptation week (initiation of treatment) at the end of the third and sixth weeks of treatment.

Propolis

Propolis powder (produced by *Apis mellifera L.* bees), added to the feed, was purchased from a commercial producer (W. Wenzel®).

Preparation of rations

The extruded commercial feed for parrots (PapagaioMix – Biotron®) was used throughout the project. In the extrusion process, after the mixing phase, the feed was separated into two fractions of 100 kg each. In one of the fractions, 500 g of propolis were added to the propolis treatment ration. The remaining fraction was used for the control treatment.

Laboratory assays

Rations tests

Three aliquots of the rations used were frozen at the beginning of the experimental period. All samples were ground in a ball mill before performing the analysis.

The analyzes of dry matter (DM) and mineral matter (ash) were analyzed according to AOAC [14].

The crude protein (CP) was calculated based on Kjeldahl method [14]. The analyzes of DM, CP and ashes were performed in duplicate at the Laboratory of Bromatology (Departamento de Melhoramento e Nutrição Animal, Faculdade de Medicina Veterinária e Zootecnia, Universidade Estadual Paulista- UNESP, Botucatu Campus, São Paulo State, Brazil).

For determination of the minerals, approximately 0.1 g of ration in triplicate was weighed and acid digestion was performed. The levels of the following minerals were determined by ICP-OES (Inductively Coupled Plasma - Optical Emission Spectrometer: OPTIMA 8300®) from the Chemistry Laboratory, Department of Chemistry and Biochemistry - UNESP - Botucatu Campus: calcium (Ca), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), sodium (Na), phosphorus (P).

Crude energy was analyzed in duplicate in an adiabatic calorimetric pump by the Laboratory of Animal Nutrition (LANA - Departamento de Zootencia, Faculdade de Ciências Agrárias e Veterinárias – UNESP – Jaboticabal Campus).

Evaluation of digestibility and consumption

The rations and feces were collected for four days, after a three-day adaptation period using all seven days the ration was marked with chromium oxide (1%), according to Rodrigues et al. [15].

For determination of digestibility, the urofecal materials were oven-dried for 72 hours and immediately frozen. At the end of the experiment, the leftovers were weighed, and the consumption calculation was based on the metabolic weight (animal weight0.75). The feces were thawed, milled in a ball mill, homogenized, and analyzed using the same methods described for the rations. The apparent digestibility coefficient (ADC) was calculated according to the formula [15]:

$$ADC = \frac{100 - 100^{*}\ (\%\ \text{ration indicator X}\ \%\ \text{faeces nutrients})}{(\%\ \text{faeces indicator X}\ \%\ \text{ration nutrients})}$$

For the calculation of energy, the following formulas were used:

$$IF = \frac{\text{Concentration of the indicator in the fecal sample}}{\text{Concentration of the indicator in the ration}}$$

AME = GE ration – GE urofecal sample * IF

IF: Indigestibility factor

AME: Apparent Metabolizable Energy

GE: Gross energy

Temperature and humidity assessment

There was a daily temperature and humidity recording (thermohygrometer Instrutemp® ITHT 2250). The data were compared to evaluate if there were differences in temperature and humidity in the two experimental periods that could affect the consumption of birds.

Statistical analysis

Statistical analysis of the data was initiated by the Shapiro-Wilk normality test and then, by checking the normality of the data, ANOVA was performed with time-repeated measures (days 4 to 7) using the PROC MIXED procedure of SAS® 9.4. All data were expressed as mean ± standard error of the mean (SEM). The Pearson correlation coefficient was used to estimate the correlation between the digestibility coefficients of the minerals. Statistical significance was defined as $p < 0.05$.

Results

Bromatological and mineral analysis of ration

The percentage of dry matter was significantly higher in the propolis ration. For the others parameters, no differences were observed between treatments. The protein and energy requirements found in the diet comply with the Association of American Feed Control Official (AAFCO) for psittacines, being higher for calcium [16, Table 1).

Table 1
Bromatological, mineral, and chromium oxide marker analysis (Cr_20_3, mean ± standard error) of the rations used in the experiments and minimum and maximum recommendations of the Association of American Feed Control Official (AAFCO, 1998).

Parameters	Control	Propolis	p	AAFCO recommendation (1998)	
				Minimum	Maximum
Dry matter (%)	92.29 ± 0.35[b]	93.70 ± 0.27[a]	0.020*		
Protein (%)	17.47 ± 0.27	17.54 ± 0.28	0.856	12.00	
Ashes (%)	4.36 ± 0.19	4.52 ± 0.05	0.438		
Energy (cal/g)	4,088 ± 27.27	4,133 ± 18.26	0.199	3,200	4,200
Ca (%)	1.46 ± 0.05	1.40 ± 0.02	0.356	0.30	1.20
P (%)	0.70 ± 0.02	0.70 ± 0.02	1.000	0.30	
Ca:P	2:1	2:1		1:1	2:1
Mg (%)	1.49 ± 0.03	1.48 ± 0.03	0.812	0.06	
Na (%)	0.31 ± 0.01	0.30 ± 0.00	0.322	0.12	
K (%)	1.00 ± 0.02	1.01 ± 0.01	0.603	0.40	
Fe (%)	0.04 ± 0.00	0.05 ± 0.01	0.429	0.008	
Mn (%)	0.02 ± 0.00	0.02 ± 0.00	0.830	0.0065	
Ni (g/kg)	2.54 ± 0.08	2.37 ± 0.08	0.154		
Zn (%)	0.09 ± 0.00	0.09 ± 0.00	0.522	0.005	
Cr_2O_3 (%)	0.03 ± 0.00	0.03 ± 0.00	1.001		

Macrominerals = Ca: Calcium, P: Phosphorus, Mg: Magnesium, Na: Sodium; Microminerals = Fe: Iron, Mn: Manganese; Ni: Nickel, Zn: Zinc. Cr_2O_3 = chromium oxide. Ca: P = Calcium and Phosphorus ratio. [a,b] Values in a row not sharing a same superscript are significantly different ($p < 0.05$). *Significant difference ($p < 0.05$) between Control and Propolis treatments.

Consumption

Consumption was calculated as a function of metabolic weight. There was no difference between feed consumption, indicating that the higher values of dry matter found in the propolis feed did not affect consumption (Table 2).

Table 2
Daily intake of dry matter, protein, and energy in the different treatments

Parameters	Control	Propolis	p
Dry matter	28.83 ± 1.06	26.99 ± 1.06	0.235
Protein	5.40 ± 0.21	5.05 ± 0.21	0.247
Energy	129.30 ± 4.70	120.16 ± 4.63	0.182

n = 10; mean ± standard error; dry matter and protein intake: (g / $kg^{0.75}$ / day); energy consumption: (kcal / $kg^{0.75}$ / day)

Correlation

The control diet had several positive correlations among the evaluated minerals. The most significant correlation was between calcium and phosphorus (r = 0.98) and the mineral that presented the most correlations was phosphorus (Table 3).

Table 3
Pearson's Correlation Coefficient (p-value) between apparent digestibility coefficients of minerals for Blue-fronted Amazon fed control ration.

	P	Mg	Na	K	Fe	Mn	Ni	Zn
Ca	0.98(< 0.00)*	0.76(0.01)*	0.57(0.08)	0.36(0.31)	0.82(0.01)*	0.86(0.00)*	0.07(0.86)	0.62(0.06)
P		0.75(0.01)*	0.68(0.03)*	0.27(0.46)	0.73(0.04)*	0.77(0.02)*	-0.04(0.93)	0.57(0.09)
Mg			0.50(0.14)	0.21(0.56)	0.61(0.11)	0.78(0.01)*	0.41(0.27)	0.62(0.06)
Na				-0.22(0.55)	0.33(0.42)	0.14(0.73)	-0.09(0.81)	0.25(0.49)
K					-0.24(0.56)	0.35(0.36)	0.47(0.21)	0.27(0.45)
Fe						0.96(0.00)*	-0.40(0.37)	0.80(0.02)*
Mn							0.18(0.67)	0.62(0.08)
Ni								-0.05(0.90)

Macrominerals = Ca: Calcium, P: Phosphorus, Mg: Magnesium,Na: Sodium; Microminerals = Fe: Iron, Mn: Manganese; Ni: Nickel, Zn: Zinc.*Significant difference (p < 0.05).

For the propolis ration, the minerals with the highest index were calcium and magnesium (r = 0.86, Table 4).

Table 4
Pearson's Correlation Coefficient (p-value) between apparent digestibility coefficients of minerals for Blue-fronted Amazon fed a ration containing propolis.

	P	Mg	Na	K	Fe	Mn	Ni	Zn
Ca	0.76(0.01)*	0.78(0.01)*	0.33(0.35)	0.53(0.12)	0.31(0.39)	0.50(0.14)	0.04(0.92)	0.17(0.65)
P		0.86(0.00)*	0.38(0.29)	0.58(0.08)	0.39(0.27)	0.57(0.08)	0.39(0.30)	-0.02(0.96)
Mg			0.40(0.29)	0.30(0.44)	0.04(0.92)	0.14(0.73)	0.13(0.75)	0.25(0.51)
Na				0.19(0.60)	-0.07(0.86)	-0.26(0.47)	-0.15(0.70)	0.65(0.04)*
K					0.56(0.09)	0.71(0.02)*	0.38(0.32)	-0.36(0.30)
Fe						0.80(0.01)*	0.56(0.12)	-0.24(0.51)
Mn							0.46(0.22)	-0.58(0.08)
Ni								-0.30(0.44)

Macrominerals = Ca: Calcium, P: Phosphorus, Mg: Magnesium,Na: Sodium; Microminerals = Fe: Iron, Mn: Manganese; Ni: Nickel, Zn: Zinc.*Significant difference ($p < 0.05$).

Coefficients of apparent digestibility of minerals and weight

The average apparent digestibility coefficients (ADC) are shown in Table 5. Iron, manganese, and nickel presented higher values in the birds that consumed propolis. There was no difference in the body weights of the birds between the treatments (Table 5).

Table 5
Apparent digestibility coefficient of minerals and weight (mean ± standard error) of Blue-fronted Amazon in the different treatments (n = 10).

Parameters	Control	Propolis	p
Minerals (%)			
Ca	54.76 ± 1.19	56.06 ± 1.17	0.443
P	54.31 ± 1.04	55.41 ± 1.00	0.454
Mg	93.73 ± 0.16^{b}	94.32 ± 0.16^{a}	0.015*
Na	82.75 ± 0.84	84.04 ± 0.83	0.287
K	78.14 ± 0.97	79.29 ± 0.95	0.405
Fe	60.95 ± 2.39^{b}	71.50 ± 2.37^{b}	0.006*
Mn	45.95 ± 2.45	46.86 ± 2.44	0.794
Ni	97.76 ± 0.14^{b}	98.74 ± 0.14^{a}	0.0001*
Zn	88.00 ± 0.64	89.48 ± 0.65	0.123
Weight (g)	447.17 ± 21.72	427.33 ± 21.72	0.527

Macrominerals = Ca: Calcium, P: Phosphorus, Mg: Magnesium, Na: Sodium; Microminerals = Fe: Iron, Mn: Manganese; Ni: Nickel, Zn: Zinc. Cr_2O_3 = chromium oxide. Ca: P = Calcium and Phosphorus ratio. ^{a,b} Values in a row not sharing a same superscript are significantly different ($p < 0.05$). *Significant difference ($p < 0.05$) between Control and Propolis treatments.

Discussion

The calcium percentages of control and propolis diets (1.46 ± 0.05% and 1.40 ± 0.02%, respectively) were higher than those recommended for psittacines (1.20%) and may have impaired the values of ADC for this mineral, which presented one of the three lowest ADCs, although the Ca: P ratio followed the recommendations of 2: 1 [16]. Calcium participates in the process of bone formation and muscle contraction and nerve conduction, in addition to blood coagulation; its deficiency affects the availability of phosphorus and vitamin D and its excess may negatively influence the absorption of the mineral itself [3, 17, 18].

Although there is no significant difference between the levels of minerals in the treatment rations, the birds that consumed propolis rations showed different correlations between the apparent digestibility coefficients (ADC) of the rations. These correlations may occur due to alterations in the synergism/antagonism of the minerals due to the antioxidant action of propolis [21–23], or by the possible alteration of the intestinal flora of the parrots, due to the antimicrobial properties of the propolis [24–27].

The three lowest rates of ADC were calcium (Ca), phosphorus (P), and manganese (Mn) in both treatments. The low ADC index for calcium possibly occurred due to the excess of this mineral in the rations. The results of the feed analysis for phosphorus and manganese were within the limits of the recommendations of AAFCO [16].

Phosphorus is a limiting element for calcium absorption [4], and it is highly important that the Ca: P balance be fit. Manganese absorption is also limited by calcium and phosphorus, which have been suggested to compete for the same binding site in mammals [3]. However, the correlations of ADC indices in both rations were positive between manganese, calcium, and phosphorus, indicating that it is not possible to dispute the binding site of these minerals in the blue-fronted amazon.

The magnesium, nickel, and iron minerals showed differences in the ADC between the treatments, and the parrots that were fed the propolis diet had higher indices. This result suggests that we have to take care about the level of inclusion of these minerals in propolis feeds, as they may present toxicity [3, 17, 18] if provided for a long period, is not necessary supplement them [17].

There was a positive correlation between the apparent digestibility coefficients of manganese and calcium for all treatments. It can be inferred that the higher manganese ADC occurred because of the high percentages of calcium in the feed. In contrast to that reported by the present study, much research suggests that calcium-limiting manganese is formed by the formation of insoluble complexes [3, 17, 28]. Haro et al. [29] did not find differences in magnesium absorption in rats fed propolis. For blue fronted amazons, the results report the possible availability of this element.

The results of the iron correlations in the ration with propolis and control ration suggest that the reduction of correlations was positive for the increase in ADC since the other minerals were not in excess (except calcium, according to AAFCO [16]) and not facilitated the absorption of iron from the diet. Captive parrots are more susceptible to iron deficiency due to feed being based on sunflower seeds, which are rich in phosphorus and reduce the availability of iron by the formation of phosphates and phytate [3, 30]. On the other hand, excess iron accumulates in large amounts in the tissues, especially in the liver, culminating in hemochromatosis - a disease that reduces liver activities - common in frugivorous and insectivorous parrots (lorikeets and cockatoos) and rare cases in the genus *Amazona sp*. [31–33]. In parrots, iron deficiencies are not observed when fed pellets or extruded diets because phosphorus levels are lower when compared to seed feeding [30].

The antioxidant and hepatoprotective capacity of propolis were evidenced in *in vitro* studies and poultry diets, as well as in studies with nickel levels in broiler diets [12, 13, 23, 34–36]. The higher nickel ADC levels in the propolis feed may be potentiators of the antioxidant action of both, forming synergism between the mineral and the apitherapy.

Regarding the weight of the animals, the variation found in the literature for captive birds is very wide, between 275 and 510g [37]. Di Santo [2] provided rations with different processing (pelleted and extruded), finding a difference between averages of weights (423 ± 0.01 g and 448 ± 0.02 g, respectively). In this work, we verified that the addition of propolis (0.5% m/m) in the birds' diet did not affect the weight of the animals, confirming the results of Silva [38], which provided three concentrations of propolis (0; 0.5 and 1%) in the diet of true parrots founding averages between 397.20 ± 86 g and 464.92 ± 15.7 g, with no difference between treatments. Thus, it is suggested that propolis does not affect the weight of birds, probably because it does not alter the energy level and grain size of the feed.

Conclusion

This study demonstrated that parrots fed with propolis had higher levels of apparent digestion coefficient of magnesium, iron, and nickel. ADC correlations among the minerals were altered when compared to the birds receiving control ration, it was inferred that this alteration promoted the absorption of minerals that could potentially improve the health and consequently the well-being of the birds.

Declarations

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All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter os materials discussed in this manuscript.

The authors have no financial or proprietary interests in any material discussed in this article.

Author's contribution statement

The authors *Silva, Teixeira, Veiga, Orsi* planned and designed the experimental study. *Silva, Vieira, Padilha, Orsi* revisioned the statistical analyses. *Silva, Teixeira, Padilha, Veiga* prepare all tables. *Silva, Vieira, Orsi* wrote the main manuscript text.

All the authors reviewed the manuscript.

Data Availability

Approved by the Ethics Committee on the use of Animals of the School of Veterinary Medicine and Animal Science (CEUA-FMVZ-UNESP Botucatu), protocol number 191/2013-CEUA.

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ATESTADO

Atesto para os devidos fins, que o Projeto de Pesquisa **"Ação da própolis na digestibilidade, bem-estar, parâmetros hematológicos e bioquímicos séricos de papagaios-verdadeiros (*Amazona Aestiva*, Linnaeus, 1758) mantido em cativeiro"**, Protocolo nº 191/2013-CEUA, do Professor Ricardo de Oliveira Orsi, desta Faculdade, a ser conduzido por **Cínthia Rio Branco da Silva** desta Faculdade, foi aprovado pela Comissão de Ética no Uso de Animais (CEUA) desta Faculdade.

Faculdade de Medicina Veterinária e Zootecnia, em 07 de fevereiro de 2014.

Profª.Ass.Drª. Maria Lúcia Gomes Lourenço

Presidente da CEUA da FMVZ, UNESP - Campus de Botucatu

Conflict of interest

There are no conflicts of interest between the authors, funding grant, university or any other parts.

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