PHYTASE ADDITION TO DIETS WITH DIFFERENT LEVELS OF METABOLIZABLE ENERGY, CRUDE PROTEIN AND AVAILABLE PHOSPHORUS TO BROILERS FROM 1 TO 21 DAYS OF AGE

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ABSTRACT

The objective of this study was to evaluate the performance and the pollutant excretion of broilers from 1 to 21 days of age fed diets with different levels of metabolizable energy (AMEn), crude protein (CP) and available phosphorus (aP), supplemented with amino acids and phytase. To evaluate the performance, 1,350 Cobb broilers at one day of age (weight of 45.5 ± 0.9g) were distributed in a completely randomized design in a 4x2+1 factorial arrangement (four levels of AMEn – 2,850; 2,950; 3,050 and 3,150 kcal/kg – and two of CP and aP – 17 with 0.34% and 19 with 0.25%, respectively, all with phytase, and an additional treatment – control, without phytase, with 21% PB, 3,000 kcal/kg AMEn and 0.46% aP), in six replications with 25 birds. To evaluate the excretion of pollutants, 270 broilers at 14 days of age were allotted in metabolic cages in groups of five during seven days, using the same statistical design. Although the use of phytase reduced phosphorus and copper excretion, it decreased weight gain and increased feed conversion ratio in all studied nutritional plans. In phytase-diets, the energetic levels linearly reduced feed intake and improved feed conversion ratio and calcium and potassium excretion. Energy reduced the nitrogen, phosphorus and zinc excretion only in 17% CP and 0.34% aP diets. Crude protein reduction in phytase-diets improved feed conversion ratio and reduced nitrogen and potassium excretion. It was concluded that phytase decreases broiler performance until the third week of age when combined with reduced levels of CP and aP in diets.

KEYWORDS: additive; enzyme; excrement quality; ideal protein; nutrition.

ADIÇÃO DE FITASE EM RAÇÕES COM DIFERENTES NÍVEIS DE ENERGIA METABOLIZÁVEL, PROTEÍNA BRUTA E FÓSFORO DISPONÍVEL PARA FRANGOS DE CORTE DE 1 A 21 DIAS

RESUMO

Objetivou-se avaliar o desempenho e a excreção de poluentes em frangos de corte de 1 a 21 dias recebendo dietas com fitase combinada com diferentes níveis de energia metabolizável (EMAn), proteína bruta (PB) e fósforo disponível (Pd) e suplementadas com aminoácidos. Para a avaliação do desempenho, 1.350 pintos machos Cobb com um dia de idade (peso de 45,5 ± 0,9g) foram distribuídos em delineamento inteiramente casualizado em esquema fatorial 4x2+1 (quatro níveis de EMAn – 2850, 2950, 3050 e 3150 kcal/kg – e dois de PB com fósforo disponível – 17 com 0,34% e 19 com 0,26%, respectivamente, todos com fitase – e um tratamento adicional – controle, sem fitase, com 21% PB, 3000 kcal/kg de EMAn e 0,46% de Pd), em seis repetições de 25 aves cada. Para a avaliação da excreção de poluentes, foram utilizados 270 pintos aos 14 dias de idade alojados

em gaiolas metabólicas em grupos de cinco, durante sete dias, utilizando-se o mesmo delineamento. O uso de fitase, apesar de reduzir a excreção de fósforo e de cobre, piorou o ganho de peso e a conversão alimentar, em todos os planos nutricionais estudados. Em rações com fitase, os níveis energéticos reduziram de forma linear o consumo de ração e melhoraram a conversão alimentar e a excreção de cálcio e potássio. A energia reduziu a excreção de nitrogênio, fósforo e zinco somente em rações com 17% PB e 0,34% de Pd. A redução da PB em rações com fitase melhorou a conversão alimentar, além de reduzir a excreção de nitrogênio e potássio. Conclui-se que a fitase piora o desempenho de frangos de corte até a terceira semana de idade quando combinada com níveis reduzidos de PB e Pd.

PALAVRAS-CHAVE: aditivo; enzimas; nutrição; qualidade das excretas; proteína ideal.

INTRODUCTION

The expansion of poultry production comes along with continuous assimilation of modern technologies, placing Brazil among the three largest chicken producers in the world. In this context, the use of diets to maximize performance and reduce the environmental impact caused by waste has been the subject of research in recent years. Thus, maximum efficiency in utilization of dietary nutrients and their reduced excretion in the environment are essential attributes to modern production systems.

The use of poultry litter in animal feed and as fertilizer has suffered restrictions for two main reasons: first, due to the appearance of bovine spongiform encephalopathy; second, because it becomes harmful to plants, groundwater and aquatic organisms, when it is applied to the soil without agronomic criteria. Therefore, it is necessary to carry out researches by means of diet manipulation to try to reduce environment problems.

Currently, various additives have been used in diets in order to improve the utilization of nutrients by animals, among them phytase has shown effect on the use of phytic acid of the food (SILVA et al., 2008). However, there are reports that this enzyme can also provide carbohydrates, amino acids and ions such as phosphate, calcium, potassium, zinc and copper (SILVA et al., 2006) and may, in this case, change energy, electrolyte and amino acid balance. This becomes more important when diets based on the ideal protein concept are used. Such concept is associated with soybean meal reduction and crystalline amino acids inclusion.

Therefore, the objective this study was to evaluate the effect of diets with reduced levels of crude protein and available phosphorus, formulated with different levels of corrected apparent metabolizable energy (AMEn), supplemented with amino acids and phytase, on performance and pollutants excretion by broilers from one to 21 days of age.

MATERIAL AND METHODS

The experiment was carried out at the Poultry Section of the Department of Animal Science of the Federal University of Lavras (UFLA), Lavras, MG, Brazil.

For performance evaluation, we used 1,350 male Cobb chicks at one day of age, with starting weight of 45.5 ± 0.9 g, randomly distributed in a masonry shed, in 54 boxes lined with wood shavings, all of them supplied with tubular feeder and pendular drinker. Water and feed were provided *ad libitum*. The experimental period lasted 20 days.

For the metabolism trial, conducted concomitantly, we distributed 270 chicks of the same strain at 14 days of age into metal battery cages (50 x 50 x 50cm), equipped with "cup" pressure drinker and feeders with wood edges to avoid waste, and excreta collection trays. The cages were placed in metabolism room under controlled environment, receiving artificial light for 24 hours. Water and feed were provided *ad libitum* during a trial period of seven days.

A completely randomized design was used in both trials, with a 4x2 +1 factorial arrangement (four levels of corrected apparent metabolizable energy – 2850, 2950, 3050 and 3150 kcal / kg – and two levels of crude protein and available phosphorus – 17 and 0.34% and 19 and 0.26%, respectively – and an additional treatment), totalling nine treatments with six replicates of 25 broilers each for performance trial and five broilers for metabolism trial. The additional treatment consisted of a control diet formulated according to the nutritional levels recommended by ROSTAGNO et al. (2000). In diets containing low phosphorus (0.24 and 0.36%) 500 FTU phytase/kg (Ronozyme - 2500 FTU/g) were added. Calcium levels were fixed in 0.8% (SCHOULTEN et al., 2003). The diets were formulated with corn and soybean meal, maintaining the ideal amino acids:lysine ratio according to the ideal protein concept (Table 1).

In the metabolism trial, we used the total excreta collection traditional method, described by

MATTERSON et al. (1965). The birds went through a four-day acclimation period to the cages, then the three-day total feces collection period started (RODRIGUES et al., 2005). During this period, excreta were collected in trays lined with plastic and separated from feathers. Ration leftover was collected and frozen until the end of the experiment, when it was sampled along with the feed and excreta for measurement of bromatological analysis, using the methodology described by SILVA & QUEIROZ (2002).

Gross energy, nitrogen, phosphorus, calcium, potassium, zinc and copper in feed and excreta were determined. For the calculation of AMEn values from the feed, we used the equations described by MATTERSON et al. (1965), and for the coefficients of nitrogen, phosphorus, calcium, potassium, zinc and copper apparent retention, we used the following formula:

$$\text{coefficient of retention} = \frac{\text{g ingested nutrient} - \text{g excreted nutrient}}{\text{g ingested nutrient}} \times 100$$

Table 1. Centesimal composition of the experimental diets[1]

Ingredient	Control diet	17% CP and 0.34% aP				19% CP and 0.26% aP			
		AMEn (kcal/kg)[1]				AMEn (kcal/kg)[1]			
		2850	2950	3050	3150	2850	2950	3050	3150
Corn	56.60	66.30	66.30	66.30	66.30	61.20	61.20	61.20	61.20
Soybean meal	35.70	24.80	24.80	24.80	24.80	30.30	30.30	30.30	30.30
Kaolin	0.40	3.89	2.77	1.63	0.49	3.69	2.55	1.41	0.28
Dicalcium phosphate	1.90	1.31	1.31	1.31	1.31	0.83	0.83	0.83	0.83
Soybean oil	3.20	0.62	1.74	2.88	4.02	1.14	2.28	3.42	4.55
Calcitic limestone	1.00	0.95	0.95	0.95	0.95	1.33	1.33	1.33	1.33
Common salt	0.49	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
L-lysine HCl – 79%	0.17	0.50	0.50	0.50	0.50	0.35	0.35	0.35	0.35
DL-methionine -99%	0.23	0.33	0.33	0.33	0.33	0.28	0.28	0.28	0.28
L-valine	-	0.20	0.20	0.20	0.20	0.10	0.10	0.10	0.10
L-arginine	-	0.16	0.16	0.16	0.16	-	-	-	-
L-threonine	-	0.08	0.08	0.08	0.08	-	-	-	-
L-isoleucine	-	0.08	0.08	0.08	0.08	-	-	-	-
Mineral Premix[2]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin Premix[3]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Choline chloride – 60%	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Salinomycin 12%	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Zinc bacitracin	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Phytase[4]	0.00	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Calculated values									
Crude protein (%)	21.1	17.0	17.0	17.0	17.0	19.0	19.0	19.0	19.0
AMEn (kcal/kg)	3000	2850	2950	3050	3150	2850	2950	3050	3150
Calcium (%)	1.00	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Available phosphorus (%)	0.46	0.34	0.34	0.34	0.34	0.26	0.26	0.26	0.26
Digestible lysine (%)	1.15	1.15	1.15	1.15	1.15	1.16	1.16	1.16	1.16
Digestible Met+cist (%)	0.81	0.82	0.82	0.82	0.82	0.81	0.81	0.81	0.81
Digestible threonine (%)	0.72	0.65	0.65	0.65	0.65	0.64	0.64	0.64	0.64
Digestible isoleucine (%)	0.83	0.72	0.72	0.72	0.72	0.74	0.74	0.74	0.74
Digestible arginine (%)	1.33	1.18	1.18	1.18	1.18	1.18	1.18	1.18	1.18
Digestible valine (%)	0.87	0.90	0.90	0.90	0.90	0.89	0.89	0.89	0.89

CP – crude protein; aP – available phosphorus; AMEn – apparent metabolizable energy corrected for nitrogen
[1] Values in kcal/kg of natural matter
[2] Supplying for kg /ration: 50 mg Zn; 20 mg Fe; 4 mg Cu; 0.2 mg Co; 75 mg Mn; 1.5 mg I
[3] Supplying for kg /ration: 6 mg vit. B2; 12.000 UI vit. A; 2.200 UI vit D3; 53 mg nicotinamida; 2.2 mg vit.B1; 3.3 mg vit. B6; 16 mcg vit. B12; 0.11mg biotin; 1.0 mg folic acid; 130 mg pantothenic acid; 2.5 mg Vit. K3; 30 mg vit. E; 120 mg anti-oxidant
[4] 2500 FTU/g of ration

We evaluated intake, weight gain, feed conversion and excretion of nitrogen, phosphorus, calcium, potassium, copper and zinc by poultry. The data were submitted to analysis of variance: first, an overall analysis of variance with all treatments was used to obtain the residue mean square; then it was used to test the factorial and perform the Dunnet test at 5% for control. The Student-Newman-Keuls (SNK) test was used, at 5% probability, for treatments in the factorial design. All tests were performed using the statistical software Sisvar (FERREIRA, 2008).

RESULTS AND DISCUSSION

Comparing the control diet with the others, we observed that the animals that received the lowest protein (17%) and energy (2850 kcal / kg) levels showed higher intake ($P < 0.05$), possibly to meet their nutritional needs (Table 2). In addition, we observed that the use of phytase in diets with reduced nutrient levels worsened weight gain and feed conversion ($P < 0.05$).

According to PINCHASOV et al. (1990), different rates of absorption of amino acids and peptides can result in inadequate amounts of amino acids at specific sites of cellular protein synthesis, which are insufficient to sustain high growth rate of chickens especially at the initial phase. In this case, the difference in digestion and availability of amino acids from intact protein, that is corn and soybean meal protein, in relation to the availability of crystalline amino acids may have influenced these results.

BREGENDAHL et al. (2002) evaluated diets with reduced levels of crude protein for broilers at the initial phase and observed that birds fed diets with reduced crude protein levels (19 and 20%) showed inferior performance than those fed diets with 23% of the nutrient. On the other hand, ROSTAGNO et al. (2002) observed that, working with normal levels of available phosphorus and without enzyme supplementation, the level of protein in the feed can be reduced up to 19%.

The present results contradict those obtained by SILVA et al. (2006), who observed no difference in performance of broilers from 1 to 21 days of age when crude protein and available phosphorus were reduced to up 17% and 0.34%, respectively, compared to the control (21% crude protein and 0.45% available phosphorus). This demonstrates the need for more studies to determine the appropriate level of nutrients to be used in diets for broilers at the early stages of development.

Considering only the rations containing phytase, no interaction was observed ($P > 0.05$) between the energy and nutrional levels in the diet. The reduction of crude protein levels associated with increased phosphorus improved ($P < 0.05$) consumption and weight gain and reduced ($P < 0.05$) feed conversion. These results suggest that wheter the use of phytase should be associated with reduced crude protein or the amount of available phosphorus in diets with 19% crude protein may have been insufficient, showing that, in this case, the enzyme did not act effectively at this level of phosphorus (0.26%).

SILVA et al. (2006), working with the same levels of protein and available phosphorus in diets with phytase for broilers up to 21 days of age, found no differences by reducing the mineral from 0.34 to 0.25% in diets with 19% crude protein; however, they observed differences in diets with 17% crude protein. These results suggest that reducing available phosphorus is more important than reducing protein, because animal performance decreases by lessening the amount of the mineral.

The energy levels of the diets did not influence ($P > 0.05$) the weight gain of broilers, but it linearly reduced ($P < 0.05$) intake ($Y = 1958$ to $0.2892X$, $R^2 = 0.99$) and feed conversion ($Y = 2.651$ to $0.0004\ X$, $R^2 = 0.98$). According to BARBOSA et al. (2008), broilers are able to regulate intake in function of energy levels of the diet, maintaining performance since an appropriate amount of nutrients is used.

Regarding nutrient excretion by broilers, comparison between control and the other rations showed that the inclusion of phytase combined with different levels of energy, protein and available phosphorus influenced ($P < 0.05$) all parameters studied (Table 3). Nitrogen excretion was lower ($P < 0.05$) in diets with the lowest crude protein level. According to BREGENDAHL et al. (2002), the reduction of one percentage unit in the protein content of the diet may decrease nitrogen excretion up to 5 to 6%. A decrease of up to 6.4% for each percentage unit reduction in dietary crude protein was observed in this study when comparing diets with 21% crude protein (control) with those containing 19%, maintaining the energy level. In the other diets containing 19% crude protein, energy level may have influenced the proper use of amino acids.

According to RAVINDRAN et al. (2000), phytic acid can form a complex with enzymes such as trypsin and pepsin, interfering with the nitrogen balance by altering protein digestion and amino

acids absorption. In this case, when using the ideal protein concept, one must take into account the amount of amino acids provided by phytase. In this paper, apparently the influence of phytase can not be observed in diets containing 19 or 21% crude protein and the same energy level.

The increase in energy levels in the diets resulted in linear increase (y = 2387.07-0.5789 X, R^2 = 0.98) in nitrogen excretion by broilers (P <0.05) only when 17% of crude protein was used. This result may be related to the lowest level of the nutrient associated with the most pronounced effect of phytase on protein digestibility at lower level and also with the lowest feed intake by broilers.

The inclusion of phytase in the diet also reduced phosphorus excretion by broilers (P <0.05). These results, associated with performance results, show that the reduction of phosphorus in the diets with the addition of enzyme is feasible to reduce the polluting power of the wastes. The improvement in digestibility and phosphorus utilization, reflected by the lowest excretion of this element, is expected because phytase hydrolyzes phytate-mineral complex, leaving phosphorus free available for absorption. In this study, phosphorus absolute excretion of broilers fed diets with phytase and reduced levels of crude protein was approximately 33.5% lower than those of broilers fed the control diet.

Table 2. Performance of broilers at one to 21 days of age fed ration with different levels of crude protein and available phosphorus and corrected apparent metabolizable energy

FEED	AMEn (kcal/kg)				Mean
	2850	2950	3050	3150	
		Feed intake (g/bird)			
17% CP and 0.34% aP	1153*	1119	1085	1056	1103a
19% CP and 0.26% aP	1120	1085	1067	1041	1078b
Mean[1]	1136	1102	1076	1049	1091
Control[2]	1085				
CV (%)	3,42				
		Weight gain (g/bird)			
17% CP and 0.34% aP	760*	755*	757*	750*	755a
19% CP and 0.26% aP	718*	723*	738*	723*	726b
Mean	739	739	748	736	741
Control	802				
CV (%)	3,41				
		Feed conversion			
17% CP and 0.34% aP	1.52*	1.48*	1.43*	1.41	1.46a
19% CP and 0.26% aP	1.56*	1.50*	1.45*	1.44*	1.49b
Mean[1]	1.54	1.49	1.44	1.42	1.47
Control	1.35				
CV (%)	2.82				

CP – crude protein; aP – available phosphorus; AMEn – apparent metabolizable energy corrected for nitrogen
*Means differ from control treatment by Dunnet test (P<0.05)
[1] Means followed by different letter in the same column differ by F test (P<0.05)
[2] Control diet containing 21% crude protein and 3000 kcal/kg of AMEn and 0.46% aP

Table 3. Nutrient excretion (mg/bird/day) by broilers at one to 21 days of age fed ration with different levels of crude protein and available phosphorus and corrected apparent metabolizable energy

Feed	AMEn (kcal/kg)				Média
	2850	2950	3050	3150	
			Nitrogen		
17% CP and 0.34% aP	732*	692*	611*	566*	650 b
19% CP and 0.26% aP	842	891	745*	799	819 a
Mean	787	792	678	683	735
Control	855				
CV (%)	7,05				
			Phosphorus		
17% CP and 0.34% aP	169*a	166*a	148*a	125*	152
19% CP and 0.26% aP	137*b	142*b	133*b	125*	134
Mean	153	154	141	125	143
Control	215				
CV (%)	7.34				
			Calcium		
17% CP and 0.34% aP	252	256	229*	193*b	233
19% CP and 0.26% aP	272	242	233*	228*a	244
Mean [1]	262	249	231	211	238
Control	266				
CV (%)	8.06				
			Potassium		
17% CP and 0.34% aP	386*	388*	372*	322*	367b
19% CP and 0.26% aP	452	443	436	424*	439a
Mean [1]	419	416	404	373	403
Control	471				
CV (%)	5.6				
			Copper		
17% CP and 0.34% aP	0.301*b	0.371*b	0.275*	0.282*	0.308
19% CP and 0.26% aP	0.395*a	0.414*a	0.289*	0.274*	0.343
Mean	0.348	0.393	0.282	0.278	0.325
Contro	0.471				
CV (%)	4.31				
			Zinc		
17% CP and 0.34% aP	3.4	3.4b	3.1*	2.6*b	3.1
19% CP and 0.26% aP	3.5	3.7a	3.2	3.3a	3.4
Mean	3.5	3.5	3.1	3.0	3.3
Control	3.4				
CV (%)	5.2				

CP – crude protein; aP – available phosphorus; AMEn – apparent metabolizable energy corrected for nitrogen
*Means differ from control treatment by Dunnet test (P<0.05)
[ab] Means followed by different letter in the same column differ by F test (P<0.05)
[1]Linear effect
[2] Control diet containing 21% crude protein and 3000 kcal/kg of AMEn and 0.46% aP

Comparing diets with phytase, we observed that the reduction of phosphorus in diets with 19% protein decreased the excretion of this element, except when the highest level of energy was used, probably due to lower feed intake. These results confirm those obtained by SILVA et al. (2008), who also observed a reduction in phosphorus excretion in diets with 0.26% of this element compared with the level of 0.34%.

The increase of the energy levels of rations reduced linearly ($y = -0.15x + 602$, $R^2 = 0.92$) phosphorus excretion ($P < 0.05$) only in diets containing 0.34% of this element. This result may be related with lower intake observed with the increase of the energy levels of the feed. However, the same linear effect can not be observed in diets with 0.26% available phosphorus, i.e., at this level of phosphorus, the reduction in consumption by the broilers with the increase of the energy levels of the feed did not affect the amount of the element excreted, suggesting that broilers are able to retain more phosphorus when it is limited in the diet. From this result, it is suggested that the amount of phosphorus commonly used in diets may be overestimated, since the broilers can adapt to conditions of reduced availability of the mineral.

As for calcium, we only observed reduction of the excretion (P <0.05) with the inclusion of phytase when the highest energy levels were used compared to control. Considering diets with phytase, the reduction of the protein associated with the highest level of phosphorus decreased excretion only in diets with 3150 kcal/kg, probably due to the lowest feed intake associated with better value for calcium:phosphorus ratio.

According to VIVEIROS et al. (2002), the use of phytase reduces the excretion of calcium and phosphorus in the environment, probably by providing greater amounts of such elements; which reduces their amounts in the diet, and consequently leads to a lower amount excreted. SILVA et al. (2008) also observed a reduction in calcium excretion when working with diets with 3000 kcal ME/kg containing phytase, regardless of crude protein levels. In this same study, the intake by the broilers did not differ between diets, suggesting a greater availability of this mineral.

Regarding potassium, there was a decrease (P <0.05) in excretion in all diets with reduced levels of crude protein. In these diets, the amount of soybean meal is lower, contributing to the lower intake of the element, thus, less excretion and greater use by broiler. Just as the calcium excretion, the inclusion of energy linearly decreased (P <0.05) potassium excretion by broiler.

The inclusion of phytase also reduced (P <0.05) copper excretion, when compared to control. The reduction of crude protein was effective in decreasing the excretion of the element only when the lowest energy level was used (2850 kcal / kg), in diets containing the enzyme. This result may be related to the greatest consumption observed in this energy level. In this case, nutrient intake was higher and the effect of reducing crude protein with the inclusion of amino acids was more pronounced. This is due to a lower complexation of this element to the dietary proteins, favoring the absorption and retention by the animals.

As for zinc, the inclusion of phytase reduced (P <0.05) excretion only in diets with the lowest level of crude protein (17%) and higher levels of energy (3050 and 3150 kcal/kg of AMEn). In addition, we observed that the energy levels reduced linearly (P <0.05) the excretion of this element only in diets with 17% crude protein. VIVEIROS et al. (2002) found that the use of phytase was able to increase retention of zinc by broilers, which suggests that the mineral also forms a complex with the phytate molecule. In this study, we only observed lower zinc excretion in diets with higher energy levels. This may be related with lower intake by broilers in these energy levels. In this case, phytase was not effective in releasing the mineral in its complexed form. On the other hand, the zinc retention efficiency may also be related with levels of copper in the diet and with the genetics used.

Based on these results, the manipulation of nutritional levels of diets containing phytase can contribute significantly to reduce the pollutants in manure; however, performance may be compromised. The results of this research suggest that more studies should be conducted regarding different nutritional plans in diets with phytase, mainly the ones using the concept of ideal protein in formulation of feed for broilers.

CONCLUSION

The use of phytase in diets with reduced levels of available phosphorus and crude protein is effective in reducing the emission of polluting elements in the excreta of broilers until the third week of age, although it reduces the weight gain of broilers and increases feed conversion.

ACKNOWLEDGEMNTS

The authors thank to the National Council for Scientific and Technological Development (CNPq) for funding the project and FAPEMIG for their support

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Submitted on: June, 24, 2010 Accepted on: September, 14, 2011.