ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF POULTRY SCIENCE

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International Journal of Poultry Science 12 (1): 45-50, 2013
ISSN 1682-8356

Effect of Phytase Supplementation on the Digestibility of Crude Protein, Amino Acids and Phosphorus of Cowpea (*Vignia unguiculata*) in Broilers

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Abstract: Cowpea contains phytate-P and other nutrients like amino acids which are poorly digested in broilers. Supplementation of such diets with microbial phytase helps to improve digestibility of these nutrients. In 2 experiments the effect of phytase supplementation on the digestibility of crude protein, amino acids and P of cowpea in broilers were investigated. Experiment One, had 6 diets in which 0, 150 or 300 g kg^{-1} cowpea replaced maize starch and 0 or 500 units of phytase enzyme (Natuphos), in a 3 x 2 factorial arrangement to determine P digestibility and performance of the birds. Experiment Two, had similar diets as in experiment 1 with the objective to determine digestibility of CP and AAs. TiO_2 was added as an indigestible marker in the diets and in each of the experiments a total of 288 1-day-old broiler chicks (Ross strain) were used. Phytase supplementation increased ($p<0.05$) digestibility of P from 55-67% and improved performance of the birds. Cowpea and its interaction with phytase had no significant effect on P digestibility, CP and AAs, except for arginine, glutamic acid and phenylalanine which were reduced ($p<0.05$). Phytase increased ($p<0.05$) digestibility of CP and cystine and reduced CP and AA losses on the basal level at zero intakes of CP and AAs. Results suggest that supplementation of cowpea based diets with microbial phytase improved the precaecal digestibility of P, CP and amino acids in broilers.

Key words: Phytase, crude protein, amino acids, phosphorus, digestibility, broilers

INTRODUCTION

Most of the P in grains and oil seed meals is in the form of phytate P. Phytate is poorly utilized by poultry because they have limited intestinal phytase. As a result, poultry diets are supplemented with inorganic P sources resulting in large amounts of P in the diets which are subsequently passed into the environment through the faeces. The activity of intrinsic phytase in diets for poultry and endogenous phytase in the digestive tract are not enough for efficient hydrolysis of phytate (Adeola and Sands, 2003; Applegate *et al.*, 2003). Attempt at finding solutions to the problem includes the commercial use of phytases which are phosphate enzymes that cleave P moieties from phytate; a practice now generally accepted (Augspurger and Baker, 2004). The beneficial effects of phytase on digestibility of P, AA and other nutrients have been reported by several authors. It has been demonstrated (Augspurger *et al.*, 2003) that P release in broiler chicks by *E. coli* derived phytase was 0.124% at 500 phytase units per kg. The efficacy of microbial phytase in alleviating the deleterious effect of P-deficient corn-soy bean meal-based diet on growth performance of chicks and weanling pigs as well as influencing the digestibility of several essential amino acids has been reported (Brana *et al.*, 2006). In contrast, others (Brandt and Allam, 1987) in their review have reported that there is some evidence in the literature that phytase supplementation has no effect on AA digestibility. Due to the high cost of conventional plant protein ingredients for production of poultry feeds in developing countries, poultry farmers use novel sources of protein as alternatives. Cowpea (*Vignia unguiculata*) is one of such protein sources with a potential for feeding poultry. Nevertheless, it has a phytic acid content of about 6.5 g kg^{-1} and P content of about 310 mg kg^{-1}. Given the ability of phytic acid in plant products to bind dietary nutrients such as minerals, proteins, free AAs and starch (Cromwell *et al.*, 1993), the occurrence of such complexes in birds when cowpea is fed will pose a limitation to its use for poultry feeding. The digestibility of P and amino acids of cowpea in broilers has not been reported. The objectives of this study was to determine 1) the digestibility of P and AAs in cowpea and 2) the digestibility of P and AA in response to phytase supplementation in broilers fed of diets containing cowpea.

MATERIALS AND METHODS

Experiment 1: Estimation of digestibility of P in cowpea

Diet formulation, housing and management of animals: Cowpea seeds obtained from the Legume Science Unit of the International Institute of Tropical Agriculture, IITA, Ibadan were oven dried at $50^{o}C$ to constant weight and milled to pass through a 2 mm sieve. Six experimental diets were formulated containing 3 levels of cowpea at 0, 150 and 300 g kg^{-1} in place of starch without or with 500 units of phytase/kg in a 3 x 2 factorial arrangement. Other ingredients were incorporated at a constant level across the diets. The premix used was P-free. Thus the variation in the level of phosphorus (2.0, 2.6 and 3.2g/kg)

Table 1: Composition (g/kg) of diets for study on the effect of phytase supplementation on P digestibility of cowpea (*Vignia unguiculata*) in broilers

Ingredients	Basal -phytase	Basal +phytase	15%CPM** -phytase	15%CPM +phytase	30%CPM -phytase	30%CPM +phytase
Maize	349.50	349.50	349.50	349.50	349.50	349.50
Soybean meal	100.00	100.00	100.00	100.00	100.00	100.00
Wheat gluten	150.00	150.00	150.00	150.00	150.00	150.00
Cowpea seed meal	0.00	0.00	150.00	150.00	300.00	300.00
Maize starch	300.00	300.00	150.00	150.00	0.00	0.00
Soybean oil	50.00	49.50	50.00	49.50	50.00	49.50
L-Lys-HCl	5.00	5.00	5.00	5.00	5.00	5.00
DL-Methionine	2.50	2.50	2.50	2.50	2.50	2.50
L-Threonine	1.80	1.80	1.80	1.80	1.80	1.80
L-Tryptophan	1.00	1.00	1.00	1.00	1.00	1.00
L-Arginine	5.20	5.20	5.20	5.20	5.20	5.20
Calcium carbonate	15.00	15.00	15.00	15.00	15.00	15.00
Salt	5.00	5.00	5.00	5.00	5.00	5.00
Premix*	10.00	10.00	10.00	10.00	10.00	10.00
Phytase	0.00	0.50	0.00	0.50	0.00	0.50
TiO_2	5.00	5.00	5.00	5.00	5.00	5.00
Total	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00
Calculated nutrients						
Energy (MJ/kg)	14.00	14.00	13.00	13.00	12.00	12.00
CP (g kg^{-1})	211.00	211.00	245.00	245.00	279.00	279.00
P-total (g kg^{-1})	2.00	2.00	2.60	2.60	3.20	3.20
Ca (g kg^{-1})	6.27	6.27	6.43	6.43	6.59	6.59
L-Lys-HCl (g kg^{-1})	10.50	10.50	12.80	12.80	15.00	15.00
DL-Methionine (g kg^{-1})	5.90	5.90	6.50	6.50	7.20	7.20
Met+Cys (g kg^{-1})	9.40	9.40	10.60	10.60	11.90	11.90
L-Threonine (g kg^{-1})	8.40	8.40	9.60	9.60	10.80	10.80
L-Tryptophan (g kg^{-1})	3.10	3.10	4.40	4.40	5.60	5.60
L-Arginine (g kg^{-1})	14.50	14.50	15.90	15.90	17.20	17.20
L-Valine (g kg^{-1})	9.80	9.80	11.70	11.70	13.50	13.50

*P-free Premix, **CPM = Cowpea Meal

in the diets was due only to the increasing level of cowpea. TiO_2 was added as an indigestible marker at the rate of 5 g/kg. The diets were pelleted without steam using 3 mm die. The gross composition of the diets is shown in Table 1. A total of 288 1-d-old broiler chicks (Ross strain) was obtained from Geflügelhof, Möckern (Möckern, Germany). They were weighed and distributed on weight basis into 36 cages with each cage of 1.7 m^2 holding 8 birds. The birds were provided with a commercial feed and water *ad libitum* for 14 days. At the end of d 14 the birds were weighed again. Each of the 6 diets was allocated randomly to 6 cages. The birds were fed the experimental diets for a further 7 days after which they were weighed to obtain their final body weights and then killed by asphyxiation in carbon dioxide. They were immediately opened, the final two-third section of the terminal ileum removed and the digesta content flushed with mineral-free water into containers and pooled on cage basis. The digesta samples were frozen, then freeze-dried and milled.

Experiment 2: Estimation of digestibility of CP and AAs in cowpea: The diet formulation used in Experiment 2 was similar to that used in Experiment 1 and is presented in Table 2 The distribution of birds into the diets and the management of the birds were similar to those described in Experiment 1.

Chemical and data analysis: Crude protein and AA analysis in the diets and digesta followed the VDLUFA procedures (Diliger *et al.*, 2004; Iyayi *et al.*, 2008). The concentration of TiO_2 in the diets and digesta were determined photometrically (Iyayi *et al.*, 2005). The contents of P in diets and digesta were determined spectrophotometrically (Pharmacia LKB Biochrom Ltd., model 80-2097-62, Cambridge, UK) as orthophosphate from filtered ash solutions, using the vanado-molybdate method (Diliger *et al.*, 2004). Precaecal digestibility coefficients of CP, P and AA in the diets were calculated using the following formula:

$$DC_{CP,\ AA\ or\ Pdiet} = 1-((TiO_{2diet} \times CP,\ AA\ or\ P_{digesta})/(TiO_{2digesta} \times CP,\ AA\ or\ P_{diet}))$$

Digestibility of CP, AA and P in cowpea was calculated using the regression model (Iyayi *et al.*, 2008). The digested quantities of CP and Aas (mg/d) and of P (g/d) based on the feed intake and their DCs were calculated and plotted against the intake of CP and AA (mg/d) and P (g/d) using Graph Pad Prism, Version 4.

Table 2: Composition (g/kg) of diets for study on the effect of phytase supplementation on CP and AA digestibility of cowpea (*Vignia unguiculata*) in broilers

Ingredients	Basal -phytase	Basal +phytase	15%CPM -phytase	15%CPM +phytase	30%CPM -phytase	30%CPM +phytase
Maize	331.5	331.5	331.5	331.5	331.5	331.5
Soybean meal	100.0	100.0	100.0	100.0	100.0	100.0
Wheat gluten	150.0	150.0	150.0	150.0	150.0	150.0
Cowpea seed meal	0.0	0.0	150.0	150.0	300.0	300.0
Maize starch	300.0	300.0	150.0	150.0	0.0	0.0
Soybean oil	50.0	49.5	50.0	49.5	50.0	49.5
L-Lys-HCl	5.0	5.0	5.0	5.0	5.0	5.0
DL-Met	2.5	2.5	2.5	2.5	2.5	2.5
L-Thr	1.8	1.8	1.8	1.8	1.8	1.8
L-Try	1.0	1.0	1.0	1.0	1.0	1.0
L-Arg	5.2	5.2	5.2	5.2	5.2	5.2
DCP	25.0	25.0	25.0	25.0	25.0	25.0
Limestone	10.0	10.0	10.0	10.0	10.0	10.0
Salt	5.0	5.0	5.0	5.0	5.0	5.0
Premix	8.0	8.0	8.0	8.0	8.0	8.0
Phytase	0.0	0.5	0.0	0.5	0.0	0.5
TiO_2	5.0	5.0	5.0	5.0	5.0	5.0
Total	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0
Calculated nutrients						
Energy (MJ/kg)	14.0	14.0	13.0	13.0	12.0	12.0
CP (g kg^{-1})	207.0	207.0	241.0	241.0	275.0	275.0
P-total (g kg^{-1})	6.5	6.5	7.1	7.1	7.7	7.7
P-available (g kg^{-1})	4.5	4.5	4.5	4.5	4.5	4.5
Ca (g kg^{-1})	9.89	9.89	10.1	10.1	10.2	10.2
L-Lys-HCl (g kg^{-1})	10.4	10.4	12.7	12.7	14.9	14.9
DL-Methionine (g kg^{-1})	5.8	5.8	6.5	6.5	7.1	7.1
Met+Cys (g kg^{-1})	9.2	9.2	10.5	10.5	11.7	11.7
L-Threonine (g kg^{-1})	8.3	8.3	9.5	9.5	10.7	10.7
L-Tryptophane (g kg^{-1})	3.2	3.2	4.3	4.3	5.5	5.5
L-Arginine (g kg^{-1})	14.4	14.4	15.7	15.7	17.0	17.0
L-Valine (g kg^{-1})	9.7	9.7	11.5	11.5	13.3	13.3

The slopes of the graphs corresponding to the digestibility of the respective nutrient were then derived. Data were subjected to routine ANOVA and factorial procedures using the SPSS 11.0 for Windows software (SPSS Inc., Chicago, IL, USA). Means were separated by Duncan Multiple Range Test (Jendza *et al.*, 2005).

RESULTS

The results of digestibility of P in the diets and cowpea are shown in Table 3. Phytase had a significant effect ($p<0.05$) on the digestibility of P in the diet and in cowpea. At any level of cowpea inclusion in the diet, the digestibility of P in the terminal ileum was higher in the phytase supplemented diets (Fig. 1). Increase in level of cowpea and interaction between cowpea and phytase had no significant ($p>0.05$) effect on P digestibility and reduction in the loss of P at the basal level. The results of performance of the birds on the P digestibility experiment are presented in Table 4. Phytase significantly ($p<0.05$) increased feed intake and body weight in the birds. Level of cowpea or its interaction with phytase had no significant effect on these parameters.

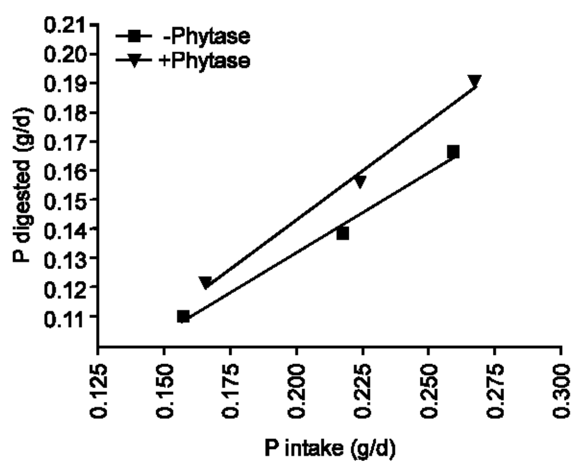

Fig. 1: Graph of P digested vs intake (g/d) in cowpea (*Vignia unguiculata*)

The results of digestibility of CP and AAs in the diets are presented in Table 5 while those in cowpea are presented in Table 6. Increase in cowpea level had no significant effect on the CP and AA digestibility in the diets except for arginine, glutamic acid and phenylalanine which were significantly ($p<0.05$) reduced. Phytase significantly ($p<0.05$) increased in the

Table 3: Digestibility of P of experimental diets and amount of P (g) digested per day up to the terminal ileum (y) depending on the daily intake (x), described by linear regression (n = 6 pens of 8 birds per treatment)

	P digestibility in the diets									
Phytase	Without phytase			With phytase			P ANOVA			
Level of cowpea (g kg^{-1})	0	15	30	0	15	30	SEM	C	P	C x P
P digestibilty	0.69[a]	0.66[a]	0.68[a]	0.73[ab]	0.74[b]	0.75[b]	0.011	0.75[ns]	0.013*	0.92[ns]

	P digestibility in cowpea	
	Without phytase	With phytase
Slope	0.55±0.051	0.67±0.053
Intercept	0.023±0.011	0.009±0.012
r^2	0.991	0.994
$S_{y.x}$	0.004	0.004
P value	0.06[ns]	0.05*

ns = not significant, * = Significant at 5% level

Table 4: Performance of birds on experimental diets of experiment 1 (n = 6 pens of 8 birds per treatment)

	Without phytase			With phytase			P (ANOVA)			
Level of Cowpea (g kg^{-1})	0	150	300	0	150	300	P SEM	C	P	C x P
Feed intake (g)	4262[a]	4519[a]	4726[a]	4354[b]	4712[b]	4866[b]	78.4	0.19[ns]	0.003**	0.93[ns]
Weight (g)	792[a]	918[a]	914[a]	815[b]	938[b]	984[b]	43.7	0.11[ns]	<0.001	0.68[ns]

[a,b]Indicate significant differences p<0.05; ns = not significant; ** = significant at 1% level; P SEM = Pooled SEM

Table 5: Precaecal digestibility of crude protein and amino acids of the experimental diets (mean; n=18)

	Without Phytase (P)			With Phytase (P)				ANOVA		
Cowpea (c)	0	150	300	0	150	300	Pooled SEM	C	P	C x P
Crude protein	0.82[ab]	0.81[ab]	0.78[a]	0.84[b]	0.83[ab]	0.83[ab]	±0.594	0.124	0.012	0.645
Alanine	0.74	0.72	0.72	0.77	0.75	0.76	±0.957	0.645	0.083	0.943
Arginine	0.89[ab]	0.88[ab]	0.86[a]	0.91[b]	0.89[ab]	0.89[ab]	±0.428	0.013	0.003	0.598
Aspartic acid	0.68	0.68	0.67	0.71	0.72	0.73	±0.948	0.948	0.026	0.857
Cystine	0.81	0.76	0.75	0.80	0.80	0.80	±0.702	0.210	0.046	0.163
Glutamic acid	0.92[b]	0.90[ab]	0.88[a]	0.93[b]	0.91[ab]	0.90[ab]	±0.392	<0.001	0.024	0.683
Glycine	0.76	0.74	0.73	0.78	0.77	0.78	±0.758	0.699	0.033	0.836
Isoleucine	0.81	0.79	0.77	0.83	0.80	0.82	±0.682	0.189	0.041	0.544
Leucine	0.83	0.81	0.79	0.85	0.83	0.83	±0.641	0.072	0.058	0.814
Lysine	0.82	0.81	0.79	0.84	0.84	0.83	±0.662	0.505	0.021	0.731
Methionine	0.90	0.88	0.87	0.90	0.89	0.89	±0.471	0.182	0.215	0.798
Phenylalanine	0.86[ab]	0.83[ab]	0.81[a]	0.88[b]	0.85[ab]	0.85[ab]	±0.613	0.006	0.008	0.687
Serine	0.80	0.77	0.76	0.82	0.80	0.80	±0.689	0.217	0.026	0.791
Threonine	0.77	0.75	0.74	0.78	0.78	0.78	±0.721	0.758	0.065	0.708
Valine	0.78	0.77	0.75	0.81	0.79	0.80	±0.790	0.667	0.037	0.573

[a,b]Indicate significant differences p<0.05 according to Turkey test

digestibility of dietary CP and AAs at the terminal ileum except alanine, leucine, methionine and threonine. Interaction between cowpea and phytase had no significant effect on CP and AA digestibility in the diets. Digestibility of CP was not significantly affected by phytase. Phytase significantly (p<0.05) increased only cystine digestibility (Table 6). Regression analysis indicates that phytase in this study affected both the CP and AA losses on the basal level (intercept) and the digestion of CP and AAs from cowpea.

DISCUSSION

The level of cowpea inclusion in the diet or its interaction with phytase supplementation did not have any effect on the digestibility of CP, AAs and P. This suggests that heated cowpea seeds can be included up to 30% in the diets of broilers. The results obtained from the 2 experiments showed that supplementation of cowpea diets with phytase is effective in improving the bioavailability of P, CP and AAs. As diets were supplemented with phytase, digestibilities of P and AAs were improved. The release of P from the phytate complex in cowpea is accompanied by release of other nutrients such as amino acids. The effect of this is improved performance as reported in this study (Table 4). As earlier reported (Jendza *et al*., 2006) there are beneficial effects of supplementing diets with phytase for nursery and growing finishing pigs. The authors' report

Table 6: Amounts of crude protein and amino acids (mg) digested per day up to the terminal ileum (y) depending on the respective daily intake (x), described by a linear regression (n = 18; parameter and SE of estimate)

	Cowpea without phytase				Cowpea with phytase				ANOVA
	Intercept	Slope	r^2	$s_{y.x}$	Intercept	Slope	r^2	$s_{y.x}$	P
Crude protein	1889±816	0.70±0.04	0.94	590	980±942	0.78±0.05	0.94	627	0.234
Alanine	60±56	0.64±0.08	0.79	43	37±60	0.70±0.08	0.81	40	0.559
Arginine	104±34	0.79±0.03	0.98	26	58±37	0.85±0.03	0.98	28	0.123
Aspartic acid	29±61	0.65±0.05	0.91	65	-23±72	0.74±0.06	0.90	71	0.254
Cystine	96±34	0.50[a]±0.09	0.64	14	8±24	0.78[b]±0.06	0.90	14	0.023
Glutamic acid	696±190	0.76±0.04	0.97	102	466±188	0.82±0.03	0.97	100	0.234
Glycine	59±42	0.65±0.06	0.87	30	20±45	0.75±0.06	0.89	31	0.300
Isoleucine	66±35	0.69±0.05	0.92	27	24±39	0.78±0.06	0.92	29	0.235
Leucine	186±79	0.68±0.05	0.92	54	106±83	0.76±0.05	0.93	56	0.300
Lysine	68±42	0.72±0.05	0.94	35	19±41	0.81±0.05	0.95	36	0.182
Methionine	47±21	0.77±0.05	0.94	12	23±21	0.84±0.05	0.94	12	0.339
Phenylalanine	101±37	0.72±0.04	0.96	29	74±43	0.78±0.04	0.95	31	0.304
Serine	90±44	0.67±0.05	0.91	32	44±48	0.75±0.06	0.92	34	0.258
Threonine	65±41	0.65±0.06	0.88	30	11±45	0.76±0.07	0.89	31	0.228
Valine	47±40	0.70±0.06	0.91	34	6±43	0.79±0.06	0.92	37	0.259

of a positive effect of the addition of phytase on feed efficiency in weanling pigs supports our results of better performance of birds on the phytase supplemented diets. Thus the addition of phytase was effective in improving the digestibility of phosphorus in the diets and in release of phytate phosphorus in cowpea.

Supplementation of the cowpea diets with phytase improved the digestibility of CP and AAs both in the diet and cowpea at the terminal ileum resulting in improved growth. In support of this, it has been reported (Kies *et al*., 2001) that supplemental phytase increased the apparent metabolisable energy of the diet. The addition of phytase to broiler diets at various production phases has been shown to increase growth and improve bone response variables (Lassen *et al*., 2001; Naumann and Bassler, 1997; Onyango *et al*., 2005). Supplementation of P-deficient corn-soya meal diets with phytase improved growth performance in broiler chicks and weanling pigs as well as influencing the digestibility of several amino acids (Pointillart *et al*., 1987). Increases of 17 and 20% in gain per bird from day 8 to 22 by supplementing a low-P diet with 500 and 1,000 FTU per kg of an evolved-*E. coli* phytase, respectively has been reported (Ravindran *et al*., 2000). Similarly, 6 and 19% improvements in gain from d 8 to 22 with 500 and 1,000 FTU of the same *E. coli*-derived phytase used in the current study per kg, respectively and 9, 14 and 14% increases in gain from d 0 to 42 in male broiler chicks with 500, 750 and 1,000 FTU of Phyzyme XP per kg, respectively have also been reproted (Rodehutscord *et al*., 2004). Adeola and Sands (2003) have reported that While supplemental microbial phytase has an improvement in utilization of plant-derived phytin P, review findings in the literature (Brandt and Allam, 1987) suggests that it may not affect amino acid digestibility. Nevertheless, differences in response of CP, AA and P to phytase supplementation can be due to the complexes present in feedstuffs, *de novo* formation of protein-phytin complexes during intestinal transient in the animal, *de novo* formation of phytin-free amino acid complexes during gastrointestinal passage in the animal and complexes involving phytin and proteolytic enzymes (Cromwell *et al*., 1993; Selle *et al*., 2000). Results of our present study suggest that the Natuphos phytase which is a 3-phytase enzyme was not only effective in improving the digestibility of P but equally improved that of CP and AA. It is important to note also that the endogenous production represented by the intercepts for P (Table 3) and for CP and AAs (Table 6) were remarkably reduced. Endogenous CP and AAs (both specific and basal) in legume-based diets is affected among other factors by the anti-nutritional factors in the legume. Since the cowpea seeds were heated before use and since heat treatment of legume seeds have been reported to reduce their antinutritional factors (Steel and Torrie, 1980; Zyla *et al*., 2000) reduced endogenous CP and AA losses in the birds was therefore obtained.

It is concluded from the present study that (i) heated cowpea can be included in the diets of broilers to a level of 300 g kg^{-1} diet (ii) supplementation of cowpea based diets with 500 units of phytase improved the digestibility of P, CP and AA with significant increase in that of cystine resulting in beneficial effect on the growth of the birds and (iii) supplementation of cowpea-based diets reduced the endogenous loss of CP, AAs and P.

ACKNOWLEDGEMENT

The author is grateful to i) the Alexander von Humboldt (AvH) Foundation for funding this study and ii) Prof M. Rodehutscord and Dr H. Kluth for their assistance in carrying out the studies.

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