

Energy utilisation and growth performance of chicken fed diets containing graded levels of supplementary bacterial phytase

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Abstract

A total of 364 female Ross 308 chicks (1 d old) were used in the present study conducted in floor pens to investigate the effects of graded levels of supplementary bacterial phytase on dietary energy utilisation and growth performance. For this purpose, four maize–soyabean-based diets were offered to the birds from 0 to 21 d of age. These included a suboptimal P negative control (NC, 3.0 g/kg non-phytate P), NC + 250 phytase units (FTU)/kg feed, NC + 500 FTU and NC + 2500 FTU. The effect of phytase activity on bird growth performance was best described as a linear relationship between increasing dose and increased feed intake ($P < 0.001$), but was quadratic for body-weight gain ($P = 0.002$) and feed efficiency ($P = 0.023$). There was no significant response ($P > 0.05$) of dietary apparent metabolisable energy (AME) to supplementary phytase. The birds fed phytase increased their retention of total carcass energy in a linear fashion ($P = 0.009$) with increased phytase dose. The efficiency of dietary AME used for overall carcass energy retention also improved ($P = 0.007$) in a linear manner with increased dietary phytase activity. Dietary net energy for production (NE_p) increased ($P = 0.047$) with an increase in phytase dose following a linear pattern, as an increase of 100 FTU increased dietary net energy by 15.4 J (estimated within the range of doses used in the present experiment). Dietary NE_p was more highly correlated with performance criteria than dietary AME, and it seems to be a more sensitive way to evaluate broiler response to phytase supplementation.

Key words: Chickens: Bacterial phytase: Net energy: Energy retention

Diet composition is a major variable in poultry production. There is a wide range of feedstuffs available to the feed industry and the decision to use a specific feedstuff is often price dependent. The price of feedstuffs depends, among other factors, on their nutrient composition and the concentration of available energy. The cost of supplying available energy accounts for about half of the cost of a broiler chicken feed⁽¹⁾. The availability of dietary energy in turn depends on the availability of carbohydrates, protein and starch, all of which may be impaired by anti-nutritive factors. Dietary phytate, a mixture of phytic acid and its salts, has been viewed as an anti-nutrient due to its ability to chelate minerals and react with starch and protein, reducing their availability for poultry^(2,3). Poultry do not produce meaningful quantities of endogenous phytase^(4,5) and, as a result, the detrimental effects of phytate in poultry diets can be ameliorated by the addition of microbial phytases.

So far, the majority of the studies evaluating the effect of phytase on available energy have been performed using the metabolisable energy system, i.e. dietary apparent metabolisable energy (AME). Although dietary AME is widely used to describe the available energy concentration in poultry feedstuffs, diets with the same AME are not necessarily used with equal efficiency when fed to poultry^(6–9). Work with exogenous phytases has shown that the improvement in performance is closely associated with destruction of dietary phytate, coupled with an improvement in the digestion and absorption of nutrients, although the influence of phytase on dietary AME *per se* has been inconsistent. Whereas some authors found an increase in dietary AME in response to phytase^(10,11), others^(12,13) did not.

Dietary net energy is the metabolisable energy of the feed corrected for losses that result from the assimilation of dietary ingredients, frequently termed the heat increment of digestion.

Abbreviations: AME, apparent metabolisable energy; CF_n, retained carcass fat; CP_n, retained carcass protein; FCE, feed conversion efficiency; FI, feed consumed from 1 d old to the end of the study at day 21; FTU, phytase units; GE, gross energy; HP_f, heat production per kg feed intake; HP_t, total heat production; K_{re}, efficiency of apparent metabolisable energy used for energy retention; NC, negative control; NE_p, net energy for production; RE_c, total energy retained in the carcass; RE_f, energy retained as fat; RE_p, energy retained as protein; WG, weight gain.

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The remaining net energy is available for both maintenance and production.

However, there is a lack of knowledge on the effect of supplemental microbial phytase on dietary net energy for production (NE_p). Pirgozliev *et al.*⁽¹³⁾ demonstrated a positive dose–response relationship between phytase and dietary NE_p in caged chickens.

Thus, the objective of the present study was to quantify the responses and inter-relationships in dietary NE_p , determined by a comparative slaughter technique, resulting from feeding graded activities of supplementary phytase to chickens reared in floor pens. Bird growth parameters and energy metabolism were also determined.

Materials and methods

Diet formulation

An *Escherichia coli*-derived phytase (Quantum™ EC 3.1.3.26; AB Vista Feed Ingredients) was used in the present experiment. A total of four experimental diets were prepared. A maize-based control diet, hereafter named negative control (NC), was formulated to be adequate in protein and energy but lower in non-phytate P content (3.0 *v.* 4.7 g/kg diet recommended by the National Research Council⁽¹⁴⁾; Table 1). The remaining three diets were the NC supplemented with phytase (250, 500 or 2500 phytase activity units (FTU)/kg diet, i.e. NC + 250 FTU, NC + 500 FTU and NC + 2500 FTU). The enzyme was added to the diets in powder form and all diets were fed as a mash. Titanium dioxide was added to the diets (5 g/kg) as an indigestible marker to enable determination of dietary AME.

Husbandry and sample collection

A total of 364 female Ross 308 chicks (1 d old) were used in the present experiment. The Animal Experimental Committee of the Scottish Agricultural College approved the study. At the beginning of the experiment, four birds from the general group, selected at random, were killed by cervical dislocation, and stored in a freezer at -20°C for analysis. The rest of the birds were allocated to twenty-four floor pens, fifteen birds in each pen, from 0 to 21 d of age. Each diet was offered *ad libitum* to birds housed in one of six pens in a randomised complete block design. The room was kept at a temperature of approximately 31°C at day 0, and this was gradually reduced to approximately 22°C at the end of the 21 d feeding period. Relative humidity was maintained at about 50%. The light regimen was 23 h light and 1 h dark. The birds were group-weighted on a per-pen basis at the beginning and at the end of the study, and the average bird weight gain (WG) and feed conversion efficiency (FCE) were determined.

At the end of the study (21 d), two birds with a body weight nearest to the pen average from each pen were transferred to one of twenty-four wire-meshed metabolism cages. The birds selected were kept in the cages for approximately 4 h and excreta were collected in the trays beneath. During this period, water was provided *ad libitum* but feed was withdrawn to minimise the contribution of undigested feed to the estimate

Table 1. Ingredient composition of the experimental control diet

Ingredients (g/kg)	
Maize	600.0
Maize gluten meal	40.0
Soyabean meal	300.0
Vegetable oil	20.0
Limestone	17.2
Monodical phosphate	7.0
Lys HCl	3.0
Met	4.2
NaCl	3.6
Vitamin mineral premix*	5.0
Total	1000
Calculated analysis (as-fed basis)	
ME (MJ/kg)	12.79
Protein (g/kg)	231
Ca (g/kg)	8.6
P (g/kg)	5.2
Non-phytate P (g/kg)	2.8
Lys (g/kg)	13.1
Met + cystine (g/kg)	9.7
Analysed values (as-fed basis)	
Protein (g/kg)	226
Ca (g/kg)	10.3
P (g/kg)	5.4
Phytate P (g/kg)	2.4
Non-phytate P (g/kg)	3.0
Ca:P	1.9

ME, metabolisable energy.

* The vitamin and mineral premix contained vitamins and trace elements to meet the requirements specified by the National Research Council⁽¹⁴⁾. All the experimental diets were designed to be low in P. The premix provided (units/kg diet): retinol, 3600 μg ; cholecalciferol, 125 μg ; α -tocopherol, 34 mg; menadione, 3 mg; thiamin, 2 mg; riboflavin, 7 mg; pyridoxine, 5 mg; cobalamin, 15 μg ; nicotinic acid, 50 mg; pantothenic acid, 15 mg; folic acid, 1 mg; biotin, 200 μg ; Fe, 80 mg; Cu, 10 mg; Mn, 100 mg; Co, 0.5 mg; Zn, 80 mg; I, 1 mg; Se, 0.2 mg; Mo, 0.5 mg.

of carcass energy retention. The birds were then weighed and killed by cervical dislocation. A comparative slaughter technique was applied to determine retention of nutrients. The carcasses of the birds, including intestines and feathers, from each cage were frozen and then minced (Hobart A 200; The Hobart Manufacturing Company Limited). The minced carcasses of the birds of each cage were pooled, thoroughly mixed and sampled, and used for following calculations. The carcass samples were freeze-dried, and carcass fat and crude protein were determined and used for following calculations based on average pen bird weight. The same procedure was applied to the carcasses of four birds taken at the start of the experiment and the data were used to determine carcass fat, protein and gross energy (GE) retention for the experimental period. It was assumed that carcass energy stored in the form of glycogen was small relative to the total carcass energy stored.

Chemical analysis

The experimental diets and the excreta were analysed for GE and titanium dioxide in order to determine dietary AME. GE was determined using a bomb calorimeter (Parr 6200; Parr Instruments Company). Titanium dioxide concentration in feed and excreta was determined using the method of

Short *et al.*⁽¹⁵⁾. Dietary Ca and total P were determined by inductively coupled plasma emission spectrometry (Optima 4300 DV Dual 150 View ICP-OE spectrometer; Perkin Elmer)⁽¹⁶⁾. The content of dietary phytate P was determined employing the method of McCance & Widdowson⁽¹⁷⁾. The N content of feed and freeze-dried carcass samples was analysed by the Kjeldahl method (Kjeltec 1035 Autoanalyser; Perstorp Analytical, Hoganas), Association of Official Analytical Chemists (AOAC) 984.13⁽¹⁸⁾. The crude protein values were obtained as $N \times 6.25$. The crude fat in the feed and carcass samples was extracted using a Soxtec system (Foss UK Limited) according to AOAC 920.39⁽¹⁸⁾.

Calculations

Dietary AME (MJ/kg) was calculated as follows:

$$\text{AME} = \text{GE}_f - (\text{GE}_e \times (\text{Ti}_{\text{feed}}/\text{Ti}_{\text{excreta}})),$$

where GE_f is the GE (MJ/kg) of the feed; GE_e is the GE (MJ/kg) of the excreta; Ti_{feed} is the concentration of titanium dioxide in the diets (g/kg); and $\text{Ti}_{\text{excreta}}$ is the concentration of titanium dioxide in the excreta (g/kg).

The total carcass GE retained in the body was obtained as the sum of the carcass GE retained as protein and fat.

The total carcass protein retention (CP_r , g/bird) was calculated as follows:

$$\text{CP}_r = (\text{N}_{21} - \text{N}_1) \times 6.25,$$

where N_{21} is the N (g) in chicken carcasses at 21 d old; N_1 is the N (g) in chicken carcasses at the beginning of the experiment at 1 d old; and 6.25 is the coefficient used to calculate the protein retained in the body.

The value of the carcass GE retained as protein (RE_p) was calculated as:

$$\text{RE}_p = \text{CP}_r \times 23.6 \text{ MJ},$$

where CP_r (kg) is multiplied by 23.6 MJ, the amount of energy in 1 kg of protein according to Okumura & Mori⁽¹⁹⁾.

The total carcass fat retention (CF_r , g/bird) was obtained similarly to CP_r as follows:

$$\text{CF}_r = (\text{F}_{21} - \text{F}_1),$$

where F_{21} is the fat (g) in chicken carcasses at 21 d old; F_1 is the fat (g) in chicken carcasses at the beginning of the experiment at 1 d old.

The value of the carcass GE retained as fat (RE_f) was obtained as follows:

$$\text{RE}_f = \text{CF}_r \times 39.12 \text{ MJ},$$

where CF_r (kg) is multiplied by 39.12 MJ, the amount of energy in 1 kg of fat according to Okumura & Mori⁽¹⁹⁾.

The total energy retained in the carcass (RE_c) was calculated as follows:

$$\text{RE}_c (\text{MJ}) = (\text{RE}_p + \text{RE}_f).$$

NE_p (MJ/kg) was calculated using the following equation:

$$\text{NE}_p (\text{MJ/kg}) = (\text{RE}_c)/\text{FI},$$

where FI is the feed (kg) consumed from 1 d old to the end of the study at day 21.

The efficiency of AME used for energy retention (K_{re}) was calculated as the RE_c divided by AME intake.

$$K_{re} = \text{RE}_c/\text{AME intake},$$

where AME intake is the FI (kg) for the experimental period multiplied by determined metabolisable energy (MJ/kg) of the diets.

Heat production

The total heat production (HP_t) of the birds from 1 d old to 21 d old, which consists of the energy for tissue retention, maintenance and the heat increment of production, was calculated as the difference between dietary AME intake and RE_c :

$$\text{HP}_t (\text{MJ}) = \text{AME intake} - \text{RE}_c.$$

The heat production per kg feed intake (HP_f , MJ/kg feed intake) was also calculated:

$$\text{HP}_f (\text{MJ/kg feed intake}) = (\text{HP}_t)/\text{FI},$$

where HP_t is the total heat production of the birds from 1 d old to 21 d old (MJ), and FI (kg) consumed.

The $\text{NE}_p:\text{HP}_f$ ratio describes the relative efficiency of the use of metabolisable energy between body energy retention and heat production, implicit that a more efficient split in energy towards production rather than heat increment is related to a higher ratio.

Statistical analyses

The observational unit was the floor pen. Statistical analyses were performed using GenStat (11th edition; Lawes Agricultural Trust, VSN International Limited). The data were analysed by ANOVA. AME intake was used as a covariate in the analysis of energy utilisation response data, because of the possible influence of variation in AME intake on the energy utilisation response criteria. Orthogonal polynomials were used to compare treatment differences for linear and quadratic relationships with increasing phytase activity (using the log phytase activity). Linear regression analysis was used to assess the relationship between supplemental phytase activity and dietary NE_p . Correlation coefficients were also generated to test for a possible relationship between the different variates. In all instances, differences were reported as significant at $P < 0.05$ and trends were noted when P was 0.05 or greater and less than 0.10.

Results

The analysed chemical composition of the basal diet is shown in Table 1. The analysed protein content was lower, although the analysed Ca content was higher than the calculated values. The contents of dietary total and non-phytate P were close to the calculated values.

Table 2 shows the data on the growth performance of chickens and dietary AME. The effect of phytase activity on the growth performance of broilers was best described as a quadratic relationship between increasing dose and increased

Table 2. Effect of the experimental diets on bird growth performance, dietary apparent metabolisable energy (AME) and dietary AME intake from 0 to 21 d of age*

	NC†	NC + 250 FTU	NC + 500 FTU	NC + 2500 FTU	SEM	L	Q
Feed intake (g/bird)	829	874	913	952	16.9	<0.001	0.096
Weight gain (g/bird)	599	618	683	730	13.45	<0.001	0.002
FCE	0.723	0.707	0.749	0.769	0.0129	0.054	0.023
AME (MJ/kg)	13.33	13.42	13.51	13.27	0.140	0.909	0.271
AME intake (MJ)	11.05	11.72	12.35	12.63	0.294	0.001	0.402

NC, negative control; FTU, phytase activity (units/kg) in diet; L, linear; Q, quadratic.
 *There were six observations per treatment.
 †NC containing 3.0 g non-phytate P/kg.

WG ($P=0.002$) and FCE ($P=0.023$) and linear with FI ($P<0.001$). Although there were no significant linear or quadratic responses ($P>0.05$) of dietary AME to phytase, there was a linear relationship ($P=0.001$) between increasing phytase activity and increased AME intake (Table 2), which was clearly related to the intake and not to the AME component.

Table 3 shows the data on the parameters describing the energy metabolism of the experimental birds. Overall, birds fed phytase tended ($P=0.059$) to increase the retention of carcass fat and increased total energy retention in a linear fashion ($P=0.009$) with increased phytase dose, which is in agreement with the growth performance and energy intake data. The efficiency of AME used for overall carcass energy retention (K_{re}) improved ($P=0.007$) in a linear manner with increased dietary phytase dose.

The NE_p content of the diet increased ($P=0.047$) with increasing phytase dose in a linear pattern (estimated within the range of doses used in the present experiment; Table 3). An increase of 100 FTU raised dietary NE_p by 15.4J ($NE_p = 5.5$ (SE 0.12) + 0.154 (SE 0.093) log FTU). The $NE_p:HP_f$ ratio also increased in a linear pattern ($P=0.006$) with increasing dietary phytase dose.

Table 4 shows the correlation coefficients of the growth and energy utilisation response criteria. WG correlated well with dietary NE_p ($r\ 0.942$; $P<0.001$), although there was no correlation with dietary AME ($P>0.05$). Similarly, FCE was correlated with NE_p ($r\ 0.764$; $P<0.001$) but not with dietary AME ($P>0.05$).

Discussion

The analysed dietary protein and Ca contents differed from the calculated values, which could probably be due to the differences between the composition of the actual ingredients that were used in the present study and the values given by the National Research Council⁽¹⁴⁾ for the same ingredients.

The experimental diets were formulated to be equally deficient in P and supplemented with graded levels of exogenous phytase to allow testing of the slope of energy metabolism responses to phytase dosage. The improvement in performance observed when phytase is fed in low-P diets has been reported quite extensively^(11,20,21). In the present study, the WG of the birds fed 2500 FTU was close to that of commercially reared birds at the same age (Aviagen Limited), and the chicks were about 15% heavier and converting feed 6.4% more efficiently compared with birds fed the NC. The NE_p values of the phytase-supplemented diets were also in the range expected for a standard poultry feed^(13,22,23). This is despite the reduction of 36% non-phytate P compared with that recommended by the National Research Council⁽¹⁴⁾. It is noteworthy that the continuing positive response of bird growth performance to supra-dosages of exogenous phytase is in agreement with previous research^(13,24), and shows that the recently recommended 500 FTU/kg seems to be much lower than the potential commercial optimum.

Further partitioning of the bird carcass into composition of gain showed that protein was responsible for the larger share of carcass energy than fat, which is in agreement with previous

Table 3. Energy metabolism of chickens (data based on the feeding period from 0 to 21 d of age)*

Variates	NC†	NC + 250 FTU	NC + 500 FTU	NC + 2500 FTU	SEM	L	Q
CP _r (g/bird)	113.5	108.1	120.9	123.8	5.17	0.669	0.119
CF _r (g/bird)	50.7	61.6	59.3	64.2	4.34	0.059	0.999
RE _c (MJ)	4.66	4.96	5.18	5.43	0.132	0.009	0.146
RE _p (MJ)	2.68	2.55	2.85	2.92	0.122	0.669	0.119
RE _t (MJ)	1.98	2.41	2.32	2.51	0.170	0.059	0.999
K_{re}	0.390	0.416	0.436	0.456	0.0110	0.007	0.165
NE_p (MJ/kg)	5.31	5.61	5.82	5.95	0.164	0.047	0.562
HP _t (MJ)	7.26	6.97	6.75	6.49	0.132	0.009	0.146
HP _r (MJ/kg)	8.31	7.89	7.56	7.09	0.158	0.002	0.031
$NE_p:HP_f$	0.632	0.716	0.784	0.850	0.0349	0.006	0.139

NC, negative control; FTU, phytase activity (units/kg) in diet; L, linear; Q, quadratic; CP_r, retained carcass protein (g/bird); CF_r, retained carcass fat (g/bird); RE_c, total carcass energy retained in a bird from 0 to 21 d of age; RE_p, carcass gross energy retained as carcass protein; RE_t, carcass gross energy retained as carcass fat; K_{re} , efficiency of dietary apparent metabolisable energy retention; NE_p , net energy for production (carcass energy retained per kg feed intake); HP_t, total heat production from 0 to 21 d of age; HP_r (MJ), heat production per kg feed intake.

*There were six observations per treatment.
 †NC containing 3.0 g non-phytate P/kg.



Table 4. Correlation matrix for the growth performance and dietary energy response criteria of broiler chickens to dietary phytase supplementation (data based on the feeding period from 0 to 21 d of age)*

	Intake	Gain	FCE	AME	AME _{int}	RE _c	NE _p	HP _t	HP _f	NE _p :HP _f	K _{re}
Intake	1.000										
Gain	0.974	1.000									
FCE	0.827	0.932	1.000								
AME	-0.087	-0.175	-0.284	1.000							
AME _{int}	0.989	0.953	0.794	0.059	1.000						
RE _c	0.999	0.968	0.811	-0.088	0.988	1.000					
NE _p	0.989	0.942	0.764	-0.049	0.998	0.990	1.000				
HP _t	-0.999	-0.971	-0.818	0.104	-0.986	-0.999	-0.988	1.000			
HP _f	-0.997	-0.975	-0.833	0.159	-0.976	-0.997	-0.978	0.998	1.000		
NE _p :HP _f	0.999	0.970	0.816	-0.068	0.992	0.999	0.993	-0.999	-0.996	1.000	
K _{re}	0.999	0.967	0.810	-0.066	0.992	0.999	0.993	-0.999	-0.996	0.999	1.000

FCE, feed conversion efficiency; AME, apparent metabolisable energy; AME_{int}, AME intake; RE_c, total energy retained in the carcass; NE_p, net energy for production; HP_t, total heat production; HP_f, heat production per kg feed intake; K_{re}, efficiency of AME use for energy retention.

* df = 9, correlation coefficients greater than 0.602 and 0.735 are statistically significant at a 5% ($P < 0.05$) and 1% level ($P < 0.001$), respectively.

reports⁽²⁵⁾. Research by Leeson & Summers⁽²⁶⁾ also showed that at an early stage of growth, broilers are depositing proportionally more carcass protein than fat, further supporting the results. No differences in carcass protein:fat ratios were observed between the treatments (data not shown).

The improvement in growth performance with phytase supplementation was coupled with an increase in dietary AME intake but not with AME *per se*, suggesting that phytase influences AME intake more through feed intake than through energy metabolisability.

The efficiency of dietary AME use for tissue energy retention is in agreement with previous reports when chickens at similar age were fed supplementary phytase^(20,27). A linear increase in K_{re} due to phytase supplementation is in accord with the higher NE_p values, which does not correlate with the effects on dietary AME. The effect of phytase addition on the improvement of NE_p but not of AME suggests that phytase enables nutrients to be digested and absorbed in the proximal parts of the small intestine when in non-supplemented diets, these nutrients may have entered the distal part of the small intestine where they may be fermented by the bacterial population. Birds do not efficiently utilise the volatile fatty acids produced⁽²⁸⁾, thus the variation in the amount of bacterial fermentation that occurs between different experiments may explain the variability in AME response to phytase in published data. The literature suggests that although phytase supplementation does not always improve dietary metabolisable energy, it usually improves intake and gain^(13,29), the former being an implicit part of AME intake, which was well correlated with NE_p. With increased AME intake, birds will have consumed more energy per d per unit of body weight, resulting in proportionately more of total intake being available for production as opposed to maintenance. The close relationship between AME intake and K_{re} is in agreement with a previous report⁽¹³⁾ and further supports this hypothesis. Thus, feeding phytase may improve energy retention as much, if not more, through increasing total energy intake, thereby increasing the supply of energy in excess of maintenance, as through increasing the efficiency of energy metabolisability. As a consequence, there was a better relationship between dietary NE_p rather than AME with bird

WG and FCE, further suggesting that NE_p is a more predictive measure for assessing the value of supplementary phytase for poultry. The present experiment has shown that phytase increases the NE_p of a diet. However, in a practical situation, an increased feed intake would also be expected, and this could further improve the economic value of the enzyme.

Birds fed the NC retained less total carcass energy and had the lowest NE_p:HP_f value compared with all the other diets. The likely interpretation for such an increase in NE_p:HP_f values is that dietary phytase will reduce the weight and the relative proportion of the gastrointestinal tract and other related organs when fed to broilers⁽³⁰⁾, and reduce endogenous secretions^(24,31). Spratt *et al.*⁽³²⁾ demonstrated that despite the fact that the liver and the gut account for approximately only 3% of the body weight of a hen, they may contribute up to 26% of the HP_t, suggesting that a relatively small reduction in the gastrointestinal tract size could account for a significant saving in maintenance energy. This suggests that birds fed phytase may have a lower heat increment, thereby allowing them to divert relatively more energy towards growth rather than maintenance.

In summary, the present results indicate that the effect of phytase on dietary NE_p was best described as a linear relationship between increasing dose and increased NE_p (estimated within the range of doses used in the present study). However, there was no significant response of dietary AME to supplementary phytase. Dietary NE_p was more highly correlated with performance criteria than dietary AME, and it seems to be a more sensitive way to evaluate broiler response to phytase supplementation. As a result, previous studies that have focused on the effect of phytase on AME alone may well have underestimated the full value of phytase. However, the effect of supplementing a high dosage of phytase to diets based on different ingredients needs further investigation, as data from the present study only indicate the potential benefits of higher levels of phytase in maize/soya-based diets that were low in P.

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