

Research Article

The Effects of Complex Enzymes on Production Performance, Egg Quality, Hatchability and Intestinal Morphometry in Khaki Campbell Duck

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ABSTRACT

A total 480 female and 80 male Khaki Campbell ducks were used to investigate the effect of enzyme complex on production performance and intestinal morphology in 24 weeks trial on wheat-based layer diets. There were four dietary treatment groups with 5 replicates in each. In T_1 group, ducks were fed basal diet and in T_2 , T_3 and T_4 enzyme complex were supplemented at the level of 0.04%, 0.05% and 0.06%. Results showed that there were no significant differences among experimental diets on body weight (BW) and feed intake. However, a significant difference (P≤0.05) on feed conversion ratio (FCR) were observed between T_4 and T_1 . Hen day egg production (HDEP) and hen house egg production (HHEP) were significantly (P \leq 0.05) improved in T₄ compared to other groups. Egg mass was significantly improved (P \leq 0.05) in T₄ than other groups. Dead germ and dead in-shells% were not affected by adding enzyme supplementation. Whereas, significant differences (P≤0.05) in fertility rate and infertile egg % were recorded between enzyme supplemented and T_1 group. Hatchability % of total eggs set was significantly different (P \leq 0.05) in T_4 whereas hatching % of fertile egg was unaffected by dietary treatments. Egg quality traits in terms of yolk weight, albumen width, shell weight, egg width, egg length, shape index and shell thickness did not differ significantly (P>0.05) among groups. Villi height (μ m) and villi area (mm²) have significantly (P≤0.05) increased in the T₃ and T₄. The results indicated that 0.06% enzyme complex supplementation in the diet may be beneficial for egg production, hatchability, intestinal morphometry but have no effect on egg quality traits in Khaki Campbel duck.

KEY WORDS complex enzymes, duck, egg production, egg quality, hatchability.

INTRODUCTION

Among livestock, ducks are around 200 times more sensitive to aflatoxins than broiler and layer chickens but the toxicosis is more harmful to the ducklings than the adult ducks (Tansakul *et al.* 2017). The ducklings particularly of Khaki Campbell are the most sensitive species to aflatoxin followed by Minikos and White Pekins. Among the cereal grains, maize is highly susceptible to aflatoxin (Mahato *et* *al.* 2019). So, considering the risk of aflatoxicosis, major duck farmers in India prefer to use wheat as the major grain source for their duck.

Wheat is major source of energy in livestock feeds but it is not so frequently used as a main source of grain in poultry diet due to lower amount of carotenoids and the presence of a group of non-starch polysaccharides (NSP), therefore, it is used to an appreciable level (5-8% of dry matter) (Mathlouthi *et al.* 2003). The water soluble pentosans form a sticky, viscous material in the small intestine. Moreover, endogenous enzymes of poultry are unable to digest NSP adequately. So, ingestion of high levels of soluble NSP leads to increased digesta viscosity and reduced nutrient digestibility and absorption (Hajati and Rezaei, 2010). Excessive NSPs in the diet may also lead to the proliferation of pathogenic intestinal microflora, such as *E. coli* and *Clostridium* spp. which initiate a mucosal inflammatory response, leading to enteric distress and suppressed gut morphological development (Choct *et al.* 2010). The problem related to NSP can be mitigated by using wheat at low levels or by the use of suitable exogenous enzymes.

Gálik and Horniaková (2010) reported that xylanase and glucanase addition in the feed of Isa Brown laying hens had a positive effect on the productivity of the birds. Similarly, Khan *et al.* (2011) found that addition of the enzyme to the basal diet significantly increased egg production, weight and mass and improved feed conversion.

The benefits of adding enzyme complex to poultry feed have been studied extensively for broilers and commercial layers (Novak *et al.* 2008) and very few studies reported in layer duck (Biyatmoko and Rostini, 2016; Hasan *et al.* 2017). A very little published information is available about the influence of enzyme complex on the hatchability of eggs in laying duck.

Considering these gaps, the objective of present study was formulated to investigate the effects of enzyme complex on egg production, egg quality, hatchability and intestinal morphology in wheat-based duck diets. To strengthen the available knowledge, this work was carried out in Khaki Campbell ducks.

MATERIALS AND METHODS

Birds, housing and environmental conditions

The experiment was conducted at Regional Exotic Duck Breeding Farm, R.K. Nagar, West Tripura. The experiment involved a total number of 480 female and 80 male Khaki Campbell laying ducks of 25 weeks of age and the ducks were randomly divided into four groups $(T_1, T_2, T_3 \text{ and } T_4)$ of 120 female and 20 male birds. Each group was subdivided into 5 replicates; each consisting of 24 females and 4 males. The experiment lasted for 24 weeks in the laying period (from 25 to 48 weeks). The ducks were housed in a deep litter system with a run area for swimming in the artificial tank. During the experimental period, the lighting schedule was maintained at 16 hours of daylight and 8 hours of darkness, at humidity 60% and a temperature at 25-30 °C. Vaccination of the experimental birds was done following the standard vaccination schedule of the farm as per duck management guideline of Central Poultry Development Organization, Hessarghata, Bangalore, Karnataka,

India. Ducks were maintained in a standard hygienic condition following all bio-security measures. The experiment followed the guidelines of the Institutional Animal Ethics Committee.

Experimental design and diets

All the ducks were fed a basal diet containing 17.87% CP and 2540 kcal ME/kg (Table 1).

Table 1	Composition	of basal	diet

Ingredient	
Composition (g/kg)	
Wheat	535
Soybean meal	166.1
Fish meal	60
Wheat bran	130
Oyster shell grit	60
Dicalcium phosphate	10
Oil	30
DL-methionine (98%)	0.4
Salt	2
Mineral premix ¹	2
Ventrimix ²	0.5
Ventribee plus ³	0.5
Choline chloride (50%)	0.1
Toxin binder	2.5
Enzyme complex	0.00
Calculated nutrient ⁴	
Crude Protein (g/kg)	178.7
ME (Kcal/kg)	2540
Ca (g/kg)	30.9
Available P (g/kg)	5.6
Lysine (g/kg)	8.4
Methionine (g/kg)	3
Methionine + cysteine (g/kg)	7.01
Threonine (g/kg)	3.52
DCAB (mEq/kg)	354
Determined analysis ⁵	
Dry matter, %	89.40
Crude fibre, %	4.33
Ash, %	10.41
Nitrogen free extract, %	62.63
Organic matter, % ¹ Provided per kg of diet: Zn: 60 mg: Mn: 90 mg: Fe:	89.59

¹ Provided per kg of diet: Zn: 60 mg; Mn: 90 mg; Fe: 110 mg and KI: 2.5 mg.
 ² Each gm contains: vitamin A: 82500 IU; vitamin B₂: 50 mg; vitamin D₃: 12000 IU and vitamin K: 10 mg.

U and vitamin K: 10 mg. Each gm contains: vitamin B_1 : 4 mg; vitamin B_6 : 8 mg; vitamin B_{12} : 40 mg;

vitamin E: 40 mg; Calcium-D-pantothanate: 40 mg and Niacin: 60 mg. ⁴ Calculated on the basis of standard values applicable under Indian Condition

(Singh and Panda, 1996).

⁵ In duplicate samples

The T_1 group was given a basal diet without adding enzyme complex. While the T_2 , T_3 and T_4 groups were supplemented with enzyme complex 0.04%, 0.05% and 0.06%, respectively. The basal diet was formulated to meet all nutrient requirements of laying duck as per the Duck management guide of Central Poultry Development Organization (Southern region), Hessarghata, Bangalore-560 088, Karnataka, India. The enzyme mixture (Zeus Biotech Private Limited, Mysore, India) included activities of xylanase (8000 U/g), phytase (50 U/g), cellulase (100 U/g), β -glucanase (1000 U/g), pectinase (1000 U/g), α -amylase (2500 U/g), protease (3000 U/g), galactosidase (1000 U/g), lipase (10 U/g) and mannanase (500 U/g). The feed was given two times daily and drinking water was provided *ad libitum*.

Determination of production Performance

In all the groups, eggs were collected daily at seven collection times: 09:00, 10:30, 12:00, 13:30, 15:00, 16:30 and 18:00 h. Egg production was recorded daily and body weight recorded initially and at the end, feed consumption was recorded weekly. Hen day egg production (HDEP) was measured daily (total number of egg produced on a day/total numbers of ducks present×100). Hen house egg production (HHEP) was measured using the formula: total number of eggs laid on a day/total number of hens housed at the beginning of laying period \times 100. Egg mass (EM) was calculated as EM= egg number / hen / day \times average egg weight (g). The value of feed conversion ratio (FCR) for each group were calculated based on egg production and feed consumption. Feed conversion was calculated by dividing the average feed intake (kg) by the average egg mass produced (average egg weight (g) \times egg production percent) expressed in kilograms of feed consumed per kilogram of egg production. Ducks are weighed at the start and end of the experiment to determine the weight gain.

Determination of the egg quality

At the end of trial, 200 eggs (10 eggs×4 treatments×5 replicates) were collected for this experiment. Egg quality traits like egg length, egg width, shell weight, egg shape index, egg shell thickness, albumin width and yolk weight were measured according to Singh and Panda (1987). After measuring the external traits, the eggs were broken open on the egg breaking stand and the contents were poured into a petri-dish to measure internal qualities. The length and width of the thick white and yolk were measured using digital vernier caliper and the mean diameters were calculated. Thereafter, yolk was gently separated from the albumin, adherent albumin was removed by rolling the yolks over a filter paper and the yolk weight was recorded. The egg shell was washed to remove the adhering albumin and after drying in oven for 24 h, their thickness was measured.

Determination of hatchability

At the end of this study, 400 eggs (20 eggs×4 treatment×5 replicates) were collected and then incubated under standard condition (100 °F temperature and 87-90% humidity) in a setter (Karamsar, Harinagar, Clock Tower, New Delhi, India) with automatic turning facility. After 7 and 14 days of incubation candling of eggs were performed to determine fertility of the developing embryo. If there was no evidence that the embryo was alive then that embryo was classified as dead. Fertility was determined as the ratio of number of fertile eggs to the number of total eggs set. Eggs from different treatments were labeled and placed in standard incubator trays in the incubator. Eggs were transferred to a hatcher (Karamsar, Harinagar, Clock Tower, New Delhi, India) at day 26. At days 28, ducklings were counted and their hatchability percentage was calculated following Anandh *et al.* (2012) and Dauda *et al.* (2014) formula:

Fertility rate %= (No. of fertilized eggs/total no. of egg set) \times 100

Hatchability %= (No. of hatched chicks/total no. of egg set) \times 100

Hatchability of fertile eggs %= (No. of hatched chicks/No. of fertilized egg set) / 100

Un-hatched eggs were broken to analyze the dead germs, dead in-shells, infertile eggs and their percentage was calculated using the following formulas:

Dead germ %= (No. of dead germs/total no of egg set) \times 100

Dead in shell %= (No. of dead in shell/total no of egg set) \times 100

Infertile egg%= (No. of infertile eggs/total no of egg set) \times 100

Intestinal morphometry

At the end of experiment, 15 ducks per treatment (3 ducks×4 treatment×5 replicates) were used to study intestinal morphometry under a high-resolution microscope with micrometry and photographic attachment (Lynx, Lawrence and Mayo Binocular Microscope). A 1 cm segment of the midpoint of the jejunum was removed, washed with physiological saline solution, and fixed in 10% buffered formalin. Each segment was then embedded in paraffin, and a 2-mm section of each sample was placed on a glass slide and stained with haematoxylin and eosin for examination. Histological sections were examined microscopically. Villus height (measured from the tip of the villus to the villuscrypt junction), crypt depth (measured from the crypt-villus junction to the base of the crypt), villus width, villus height to crypt depth ratio, villus height to villus width ratio and villus surface area:

 $[(\pi \times mh \times h) + (\pi \times mh/2) 2]$

Where:

mh: width at the mid-villus height.

h: villus height (Law et al. 2007).

Villi length and width were measured from 5 villi per duck and only the complete, vertically oriented villi were measured. Jejunum was of particular interest because it is a major site of nutrient absorption in poultry (Horn *et al.* 2009).

Statistical analysis

The data were analyzed using one-way ANOVA (SPSS,). The results were expressed as the mean and pooled standard error of mean. The specific P-values were mentioned in the text for where there was a significant difference found.

RESULTS AND DISCUSSION

A summary of production performances is presented in Table 2. No statistically significant differences (P>0.05) were recorded among the groups with respect to body weight and feed intake/duck/day. But there was a significant difference (P \leq 0.05) on FCR between T₄ and T₁ group whereas no differences were observed between T₂, T₃ and T_1 group. In the present study, the improvement in FCR was recorded in the T₄ group. The HDEP and HHEP were significantly (P \leq 0.05) improved in T₄ compared to T₁ and other groups. But there was no difference (P>0.05) on egg production between T1, T2 and T3 groups. At higher dose of enzymes supplementation, the overall average egg production was 15% higher than T₁ group. No statistical differences (P>0.05) were observed among treatment groups with respect to average egg weight. However, egg mass (g/bird) was significantly improved in T_4 than T_2 and T_1 groups.

The present findings on body weight are at par with Narasimha *et al.* (2013) and Pandian *et al.* (2017) who reported that enzyme supplementation in barley and wheatbased layer hen rations did not affect body weight (BW) gain but in contrast with the findings of Chakravathi and Mohan (2014) and Hasan *et al.* (2017) who reported increased BW in laying hen.

These results corroborate the study conducted by Narasimha *et al.* (2013), Filho *et al.* (2015) and Resende *et al.* (2017) who observed that the addition of a dietary enzyme complex did not influence the feed intake of commercial laying hens. However, Gentilini *et al.* (2009) indicated a significant reduction in the feed intake of laying hens fed diets containing enzyme complex. Wu *et al.* (2005) reported increase in egg production but feed intake did not change (P>0.05) in enzyme supplemented commercial leghorn hen which support our findings. NSP content of diet reduces the passage rate of feed, increases proliferation of microflora in the small intestines and utilize carbohydrate and protein as well as compete with the host for nutrients

(Acamovic, 2001). The significantly improved feed conversion of the birds fed enzyme may be a consequence of decreasing microbial colonization in the gut, thereby improving the availability of nutrients. Such enzyme induced improve feed conversion caused significant increase of egg production, egg weight and at the same times the insignificant differences in feed consumption among treatments.

Biyatmoko and Rostini (2016) and Pandian *et al.* (2017) observed similar increased egg production in layer chicken fed with fibre degrading enzymes. In contrast, Yoruk and Bolat (2003) using β -glucanse-xylanse-amylase in maize-barley based layer rations observed no effect on egg production by enzyme supplementation. Active enzyme in the present enzyme complex might have facilitated breaking down water soluble β -glucans and arabinoxylans (pentosans) and other viscous polysaccharides and improved mobility of feed in digestive tract. Moreover, enzymes can break down the cell walls of the feed particles, so it would be easier to digest, improve nutrient availability and led to the results of increased egg production (Mathlouthi *et al.* 2003).

Viana et al. (2011) and Narasimha et al. (2013) observed that enzyme supplementation in layer hen rations did not have any effect on egg weight which support our findings. The results are similar to the finding of Jalal and Scheidele (2001) who observed significant effect ($P \le 0.05$) on egg mass in laying hen. In contrast, Torki et al. (2014) reported no effect on egg weight and egg mass of laying hen fed whole date waste with β -mannanase-based enzyme preparation. Um et al. (1998) also reported decrease egg weight of laying hen fed different level of wheat and multi carbohydrases preparation. But in contrary, Adubados (2011) observed increase in egg weight by enzyme supplementation in layer hen. Whereas, Biyatmoko and Rostini (2016) found increased egg weight of Alabio duck fed protease enzyme in basal diet. The effect on egg weight in the present experiment is supported by the findings of non-significant effect on yolk weight between the experimental groups. Egg mass was determined by two components, egg weight and egg production. In the present study, the similar trend of egg mass and egg production indicates that variability in egg mass was mainly due to differences in egg production.

The results of egg quality traits are presented in Table 3. Yolk weight, albumen width, shell weight, egg width, egg length, shape index, shell thickness was not significantly (P>0.05) influenced by dietary treatments. That might be due to the level of enzyme complex which was not sufficient to promote degradation of NSPs and to improve nutrient utilization.

Similar to our result, previous research also revealed that enzyme complex did not affect egg quality parameters (Geraldo *et al.* 2014; Resende *et al.* 2017).

		Table 2	Effect on	production	performance of	of duck	received c	omplex enzy	yme
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Group	Complex enzyme added	Average body weight (kg)	Feed In- take/duck/day	FCR (kg in- take/kg egg)	HDEP	HHEP	Egg weight (g)	Egg mass (g)/duck
T_1	0	1.663	125.38	7.66 ^b	41.11 ^a	33.45 ^a	59.30	21.02 ^{ab}
T_2	0.04%	1.671	125.00	7.29 ^b	38.99ª	31.95 ^a	58.58	19.40 ^a
T ₃	0.05%	1.712	127.00	6.63 ^b	41.77 ^a	33.04 ^a	58.76	20.76^{ab}
T_4	0.06%	1.696	128.63	3.34 ^a	56.14 ^b	47.72 ^b	60.27	28.68 ^b
	P-value	0.015	0.73	0.48	2.50	0.63	0.33	0.84
	SEM	NS^1	NS	P≤0.05	P≤0.05	P≤0.05	NS	P≤0.05

FCR: feed conversion ratio; HDEP: hen day egg production and HHEP: hen house egg production.

The means within the same column with at least one common letter, do not have significant difference (P>0.05).

SEM: standard error of the means.

NS: non significant.

 Table 3 Effect on egg quality of ducks received complex enzyme

Group	Complex	Yolk	Albumen	Shell	Egg width	Egg length	Shape	Shell thickness
Group	enzyme added	weight (g)	width (mm)	weight (g)	(mm)	(mm)	index (%)	(µm)
T ₁	0	19.53	133.68	5.48	42.31	59.24	0.71	0.37
T_2	0.04%	19.64	133.85	5.76	42.39	57.74	0.74	0.39
T ₃	0.05%	19.90	133.19	5.23	42.45	58.18	0.73	0.39
T_4	0.06%	19.75	139.07	5.37	42.71	58.20	0.74	0.37
	SEM	0.21	1.10	0.09	0.10	0.39	0.01	0.01
	P-value	NS	NS	NS	NS	NS	NS	NS

SEM: standard error of the means

NS: non significant.

 Table 4 Effect on hatching performance of duck received complex enzyme

dded fertile egg 34.53	total eggs 20,77 ^a	% 61.23 ^a	• • • - - d	%	%
	20.77 ^a	61 22ª	• • • • •		
		01.25	38.77 ^d	7.93	27.90
6 35.57	25.05 ^{ab}	69.66 ^b	30.34 ^c	6.77	28.49
6 48.11	38.44 ^{bc}	78.48°	21.52 ^b	5.96	24.09
⁶ 49.15	42.49°	86.65 ^d	13.35 ^a	3.05	26.68
3.45	3.32	3.05	3.04	1.15	3.21
ie NS ¹	P≤0.05	P≤0.05	P≤0.05	NS	NS
	6 48.11 6 49.15 1 3.45 ne NS ¹	6 48.11 38.44^{bc} 6 49.15 42.49^{c} 1 3.45 3.32 1e NS ¹ P ≤ 0.05	6 48.11 38.44^{bc} 78.48^{c} 6 49.15 42.49^{c} 86.65^{d} 1 3.45 3.32 3.05	648.1138.44 ^{bc} 78.48 ^c 21.52 ^b 649.1542.49 ^c 86.65 ^d 13.35 ^a 13.453.323.053.041eNS ¹ $P \leq 0.05$ $P \leq 0.05$ $P \leq 0.05$	648.1138.44bc78.48c21.52b5.96649.1542.49c86.65d13.35a3.0513.453.323.053.041.151eNS1P ≤ 0.05 P ≤ 0.05 P ≤ 0.05 NS

The means within the same column with at least one common letter, do not have significant difference (P>0.05). SEM: standard error of the means.

NS: non significant.

In contrast, Mohammed *et al.* (2010) found a negative correlation between enzyme supplementation and shell thickness while Elemenawey *et al.* (2010) observed a positive correlation. Torki *et al.* (2014), reported that enzyme supplementation did not affect egg shell thickness and shell weight in laying hen. Shape may contribute in solidity of the egg and may affect gas transfer (Bain, 1991). In the present study, egg shape index did not affect by enzyme supplementation. So, this observation suggested that enzyme supplementation had no effect on the formation of egg shape.

The effects of enzyme complex on hatching traits are presented in Tables 4. The results indicated that dead germ% and dead in shell% were not affected (P>0.05) by enzyme supplementation in treatment groups. However, lowest dead germ and dead in shell % were recorded in T₄. Significant differences (P \leq 0.05) in infertile egg % were recorded between treatment and T₁ groups. The lowest infertility was recorded in T₄ followed by T₃ and T₂ group. There was significant difference (P \leq 0.05) on fertility rate between treatment and basal diet group. Fertility rate was highest in T_4 followed by T_3 and T_2 groups. Hatchability % of total eggs was significantly different (P \leq 0.05) in T_4 groups as compared to T_1 and T_2 . The highest hatchability% of total eggs was recorded in T_4 and the lowest was recorded in T_1 group.

Compared to T_1 , enzyme supplementation increased hatchability % by around 1.21, 1.85 and 2.05 times in T_2 , T_3 and T_4 , respectively. Hatching % of fertile egg was not significantly (P>0.05) different among the treatment and basal diet group. But there was increasing trend of hatching% of fertile egg between the T_2 , T_3 and T_4 groups. Highest hatching % of fertile egg was found in T_4 followed by T_3 and T_2 .

A very few published information is available about the influence of enzyme complex on the hatchability of eggs in laying duck. The reduction in infertile egg % in the present study might be due to the production of healthy eggs with supplemented enzyme complex which can enhance nutrients absorbance necessary for production performance. The present findings are in line with the findings of Awad *et al.* (2014) and Hasan *et al.* (2017).

Group	Complex enzyme added	Villi length (µm)	Villi width (µm)	Crypt depth (µm)	Villi area (mm²)	Villi height/crypt depth (µm)	Villi height/width (µm)
T_1	0	1853.50 ^a	282.75	239.00	1.89 ^a	8.20	6.69
T_2	0.04%	1576.75 ^a	283.00	187.75	1.64 ^a	10.76	5.81
T_3	0.05%	2586.75 ^b	347.75	261.25	3.12 ^b	10.36	7.56
T_4	0.06%	2699.25 ^b	347.25	286.00	3.23 ^b	9.67	7.90
	SEM	133.87	16.36	18.12	0.22	0.92	0.35
	P-value	P≤0.05	NS^1	NS	P≤0.05	NS	NS

Table 5 Intestinal morphometry of ducks received complex enzyme

The means within the same column with at least one common letter, do not have significant difference (P>0.05).

SEM: standard error of the means NS: non significant.

In contrary, Malekian *et al.* (2013) reported that supplementing broiler breeder diets with multi enzyme and phytase did not make any improvement in hatchability.

The micrometric measurements of jejunal sections of different treatments are presented in Table 5. It was seen that villi height (µm) and villi area (mm²) have been significantly (P \leq 0.05) increased in the T₃ and T₄ than T₁ and T₂. Whereas, villi width, crypt depth, villi height/crypt depth and villi height/width were similar but there was an increasing trend in T_3 and T_4 compared to T_1 . Our results are in line with Salleh et al. (2005) who reported that the combination of phytase and xylanase increased villus height in the jejunum. Iji et al. (2001) found that the addition of xylanase to wheat-based diets had no effect on crypt depth in jejunum of broiler. In contrast, Yaghobfar et al. (2007) reported addition of glucanase and xylanase in layer hen significantly reduced villus height, villus width, crypt depth and villus height:crypt depth ratio in the duodenum and jejunum of small intestine. Ayoola et al. (2015) reported that dietary β -mannanase supplementation improved the jejunum tip width, base width, surface area and villi height/crypt depth ratio in turkey and broiler, respectively. Luo et al. (2009) reported that tall mucosal villi increase the surface area available for nutrients absorption which support our present findings. In the present study, improved apparent nutrient utilization which reflect in better production performance could likely due to improved villi length and villi area of ducks received complex enzymes.

CONCLUSION

Because supplementation with enzyme complex, improved FCR, hen day egg production, hen house egg production, egg mass, hatching %, fertility rate, villi length and villi area, it can be concluded that effect of adding enzyme complex was positive at the dose rate of 0.06%. But there was no effect on egg quality traits of Khaki Campbell duck. However, further research on diverse dosages on production performance in laying Khaki Campbell duck is essential to attain more comprehensive results.

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