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Original Article

Efficacy of Feed Ingredient with Probiotics Properties, on the Growth Performance and Health of Giant White Bouscat Red Eye Rabbits

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Abstract

This study aims to evaluate the efficiency of a feed ingredient with probiotic properties (FIPP) on growth performances of Giant White Bouscat red eye rabbits. The experiment was carried out during 56 days on 150 weaned rabbits in Cuniglo farm in Southern of Benin. Rabbit had 35 days-old and weighted on average 669.94 ± 69.55 g. The experimental design was a randomized complete block with five dietary treatments (TF0, T0, T1.5, T3 and T4.5) and six replicates per treatment. TF0 rabbits were fed with the control diet and received antibiotics, T0 rabbits were fed only with the control diet; T1.5, T3 and T4.5 rabbits were fed with the control diet supplemented with 1.5%, 3% and 4.5% of FIPP respectively. The results showed that the rabbits fed TF0, T1.5 and T3 had the best weight performances compare to those fed T0 and T4.5% dietary treatments. Hematological, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) (p=0.058) and lymphocyte (LYM) are improved with the supplementation of FIPP. The lowest feed cost was in T3 (1815 FCFA/kg weight gain) followed by TF0 (1812 FCFA/kg weight gain); T1.5 (1860 FCFA/kg weight gain); T0 (2144 FCFA/kg weight gain) and T4.5 (2859 FCFA / kg weight gain). The economic feed efficiency had the same trends as feed costs. We can conclude that FIPP at concentrations of 1.5% and 3% had an effect on growth performances and on blood characteristics of rabbits.

Keywords: Rabbit, feed, growth, probiotic, hematology, efficiency.

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Introduction

The effect of food on the performance value of livestock is closely linked to the quality and importance of the microbial burden of the host animal, such as a digestive tract and its environment (Chafai *et al.,* 2006). But, the microbial load is also involved and especially in the digestive disturbances in young rabbits in the case of a deficiency of fibers or inoculated with a pathogen (Licois *et al.,* 1990).

The antibiotics used in livestock as a growth factor or against pathogenic bacteria, alter inevitably the digestive flora, since their role is to inhibit the growth of certain bacterial families (Gidenne *et al.,* 2007). Moreover, antibiotics can, if the waiting period is not respected, leave hazardous residues in animal products for the consumer and can cause hypersensitivity accidents or poisoning, while promoting the selection of bacteria resistant to subsequent treatments (Bada-Alambedji *et al.,* 2004). The prohibition of antibiotic growth promoters in animal feed led the livestock sector to implement alternative strategies such as the use of non-therapeutic substitutes (prebiotics, probiotics, symbiotic and phytogenic or phytobiotiques) (Alloui, 2011). FAO/WHO has adopted the definition of probiotics as “Live microorganisms which when administered in adequate amounts confer a health benefit on the host” (FAO/ WHO, 2002).

According to Guerra *et al.,* (2007), the use of probiotics based on lactic acid bacteria including *Pediococcus acidilactici* NRRLB-5627, *Lactococcus lactissu bsp. Lactis* CECT 539, *Lactobacillus caseisu bsp. Casei* and *Enterococcus faecium* CECT 4043 at doses of $2,6x10^{10}$, $1.4x10^{10}$, $1.3x10^{10}$ and $1.1x10^{10}$ CFU/g enabled a significant improvement of 1.6 kg weight gain and a reduction in the consumption index 0.1 on average between 21 and 63 days of age. But although probiotics and antibiotics are widely used in rabbit breeding, there is very little scientific information recently published regarding the effects on cecal microflora. However, many studies have analyzed the potential effect of probiotics on mortality and growth of the rabbit or its fermentation profile (Maertens *et al.,* 2006).

In Benin, probiotic qualities were noted on traditional beer ferments "tchoukoutou". Indeed, Kayodé *et al.,* (2012) showed that extracts of these enzymes inhibiting strains of bacteria are resistant to some antibiotics such as methicillin. In the same vein, Houndonougbo *et al.,* (2011) and Gnikpo *et al.,* (2014) showed that crude residue "tchoukoutou" in the diet of broilers and a stabilized product of these ferments "food ingredient to probiotic properties" in the Clarias gariepinus feeding helped improve performance livestock of these species.

The domestic breeding system is multi-species, the study of the effect of this "food ingredient with probiotic properties" on rabbits, which is high across Benin including 43 breeding area of 77 (Kpodekon *et al.,* 1993) is essential. The "food ingredient to probiotic properties" improves growth performance and health rabbits.

The objective of the present study was to assess whether the proposed food ingredient with probiotic properties, improves growth and health performance of Giant White Bouscat red eye rabbits.

Materials and Methods

The experiment was conducted between July-August 2014, on a private farm "CUNIGLO" located at Abomey-calavi in Benin. Two types of materials were used, the rabbits of the local population of Giant white Bouscat red-eye and formulated diet (Table 1) containing the Food Ingredient with Probiotic Properties (FIPP) incorporated in the wet phase.

Table 1: Food formula.

Ingredients	Percentage	Nutriment / Energy	Percentage / « kcal/kg »
Maize	22	Crude fiber (%)	9,34
Oilcake palmist	30	Crud fat (%)	7,51
Cottonseed meal	7	Digestible energy (kcal/kg)	2665
Soyabean meal	9	Crude proteins (%)	17,62
Wheat bran	28,6		

Oyster shell	1,7
Salt	0,3
Lysine	0,1
Methionine	0,1
Premix for broiler	0,2
Phosphate bicalcique	1
Total	**100**

The experimental design was a randomized complete block with 5 treatments and 6 replicates. Five types of food treatment were: TF0 was the food formulated without FIPP but the experimental rabbit receive antibiotics; T0, T1.5, T3 and T4.5 were food formulated with 0%, 1.5%, 3% and 4.5% of FIPP respectively and without antibiotics.

A total of 150 rabbits from 35 days of age, with 669.94 ± 69.55 g were divided by treatments with a total 30 repetitions of 5 post-weaning rabbits. Veterinary treatment applied during the experiment consisted of anticoccidial and vitamin. The antibacterial treatment was applied only to rabbits fed TF0 food.

Food Ingredient with Probiotic Properties

The food ingredient with probiotic properties (FIPP) (Table 2) used was a stabilized product of sorghum flour and the traditional ferment "kpete-kpete" (Table 2) which was the traditional starter used for the fermentation of “tchoukoutou” one of the Beninese sorghum beers. FIPP was produced according to the method described by Deh (2009): sorghum grains were husked and ground. The semi-solid paste resulting from humidification of dry sorghum flour was pasteurized and inoculated with "kpete-kpete" at 10 %. The inoculated paste was fermented for 24 h. This fermented paste was used directly without drying as an additive in formulated feed which passes through the granular.

Table 2: Characteristics of the food ingredient probiotic properties in the wet phase, dry phase and "kpete-kpete".

Variables	**Food ingredient probiotic properties wet**	**Food ingredient probiotic properties dry (Djègui, 2012)**	**Kpete-kpete (Hounhouigan, 2007)**
Dry matter (%)	50,25±0,97	84,39±0,99	10,7
pH	3,89±0,02	4,15±0,09	3,4
Lactic acid bacteria (Logcfu/g)	10,44	7,95±0,76	8,35 ± 0,10
Yeasts (Log cfu/g)	9,83	7,6±0,10	8,26 ± 0,26

Statistical Analysis

The data were analyzed with SAS software (Statistical Analysis System, 2006) by the procedure of the generalized linear model (GLM). The mathematical equation of the model was:

$Yjl = \mu + Kj + \varepsilon jl$.

Yjl = Observation of the dependent variables;

μ = overall average;

Kj= Fixed effect of dietary treatments;

εjl = Random residual error.

Averages were calculated and compared in pairs by the Student t test.

Results and Discussion

Zootechnics Parameters

The supplemented diet with Feed Ingredient with Probiotics Properties (FIPP) did not show significant differences between feed intake (FI), live weight (LW), daily weight gain (DWG) and feed conversion ratio (FCR) ($p>0.05$). However, rabbit fed with FIPP increased FI (13.22%; 10.57%; 12.32% and 18.22% for T1.5; T3, T4.5 and TF0 respectively); LW (11.22%; 14.31%; 9.73% and 12.91% for T1.5; T3, T4.5 and TF0 respectively); DWG (20.73%; 27.34%; 15.71% and 23.46% for T1.5; T3, T4.5 and TF0 respectively) and decreased FCR (17.46%; 23.11% and 15.51% for T1.5; T3 and TF0 respectively) compared with at 13th week of age (Table 3).

Table 3: Effect of dietary supplementation with Feed Ingredient with Probiotics Properties on growth performance of rabbit.

	T0	T1.5	T3	T4.5	TF0	P
LW at 35 d (g)	663.7±57.9	667.9±70.5	662.4±71.4	684.1±92.8	671.6±75.2	0.9
LW at 63 d (g)	1150.8±152.7	1179.5±227.2	1192±189.5	1232.2±250.6	1226.9±188.5	0.9
LW at 91 d (g)	1401.5±310.3	1558.8±136.4	1602±209.4	1537.9±431.1	1582.5±211.4	0.3
FI 57-63 d (g /w)	326±95.6	446±136	421.5±86.9	393.8±139.6	372±83.4	0.4
FI 84-91 d (g /w)	473.4±162	494.6±87,9	521.5±106.3	483.5±134.8	544.7±50	0,8
CFI 35-63 d (g)	1506.4±286.6	1693.5±277.0	1520.6±270.1	1637.5±424.7	1621.8±295.7	0,8
CFI 35-91 d (g)	3083.4±662.5	3490.9±480.4	3409.3±558	3463.4±802.9	3645.3±668.3	0,7
DWG 35-63 d (g/d)	17.4±3.8	18.3±6.0	18.9±5.9	19.6±6.3	19.8±4.2	0.9
DWG 35-91 d (g/d)	13.2±4.8	15.9±2.2	16.8±3.5	15.2±6.7	16.3±2.8	0,6
FCR 35-63 d (g feed/g gain)	3.1±0.4	3.6±1.3	3,0±0.6	3.1±0.6	2.9±0.2	0,5
FCR 35-91 d (g feed/g gain)	4.8±2.0	3.9±0.3	3.7±0.3	5.5±4.3	4.0±0.6	0.6

LW = Live weigth, FI = Food intake, CFI= Cumul food intake, DWG= Daily weigth gain, FCR= Feed conversion ratio.

Although TF0 revealed the highest value of FI (3645,3± 668.3 g/rabbit for 8 weeks) it was T3 that showed the best FCR (3.66±0.32). This result is in agreement with those of Ben Rayana *et al.,* (2009), who used a probiotic Biosol (association: Betafin BT and *Anterococcus faecium*) and Pascual *et al.,* (2008) who used a Toyocerin (*Bacillus cereus* var. *toyoi*) reported an increase in average daily feed intake varying between 3.13% and 28.5%. This could be explained by the fact that rabbits fed with FIPP didn't get much energy from foods or valorized early foods energies. According to Montessuy *et al.,* (2009), daily feed intake significantly decreases (5.6 g/d/rabbit) with increasing food's energy level.

Because of the increase in DWG and the decrease in FCR, FIPP could be used in rabbit as a growth promoter to enhance zootechnic parameters. Different mechanisms proposed for body weight gain may either be due to the fact that organic acids reduce colonization of bacteria in intestine and decrease the thickness of mucosa layer, increase villus height and function of secretion, digestion and absorption of nutrients that leads to the improvement of weight gain of broilers (Sohail *et al.,* 2015 and Loddi *et al.,* 2004). But going from T3 to T4.5, there is increased value of FCR. The contribution of FIPP in the diet of rabbits induced an increase in the diversity of bacteria in the digestive flora and the production of organic acids from the diet of carbohydrates (acetic acid, propionic acid, butyric acid). With the increase in the concentration of FIPP, these fatty acids may reduce the production of hydrogen peroxide and diacetyl which are antibacterial substances (Salminen, 1999; Krehbiel *et al.,* 2003; Grajek *et al.,* 2005). The FIPP also contain yeast (7.6 to 9.83 log cfu / g). The phenotypic characterization of yeasts isolated from "kpete-kpete", a traditional starter of a Benin sorghum beer reveled that through the 49 yeasts found belong to five genera and seven species of yeasts and seventy-one percent (71%) of the isolates were identified as *Saccharomyces cerevisiae* (Djegui *et al.,* 2015). According to Campos-Morales (2014) yeast supplementation impaired (P<0.01) the digestibility of nutrients. According to Renouf (2006), certain microbial metabolisms are the source of compounds that may affect the health of the consumer. This is the case of biogenic amines produced by decarboxylation of some amino acids. These volatile phenols and biogenic amines can be the cause of the increased value of FCR with increasing dose of FIPP from 3% to 4.5% in the diet. But T3 indicates the lowest value of FCR. This reduction in FCR could be explained by the increasing diversity in lactic acid bacteria that improve the valuation of food intake.

Mortality and Blood Parameter

Statistical analysis revealed no significant difference (P>0.05) between the different mortality rates and blood parameters with different treatments. Moreover, the high value of Red blood cells (RBC), haemoblobin (HGB), haematocrit (HCT) observed with rabbit which were not fed with FIPP could suggest that rabbits with neither antibiotic nor FIPP are stimulating their own organism to fight against pathogenic bacteria. This could also be explained by a vitamin deficiency (B12 and / or B9) due to poor absorption in the gut. By cons, MCH and MCHC high in treatments with FIPP indicate better assimilation of iron and nutrients. It also reflects a good transport of O_2 and CO_2 in the body of rabbits (Noureddine *et al.*, 2011). According to Capcarova *et al.*, (2013, 2009); Tidjane *et al.*, (2008), the decreasing WBC, RBC, Hb often indicated a disruption of erythropoiesis (process which produces red blood cells) or an increased in the in the destruction of RBC when the animals are exposed to any metal (bendiocarb, carbamate, mercury, silver). However, we notice that the incorporation of FIPP had tendency to reduce the mortality to 33.33%; 16.68%; 25% and 25% with T1.5; T3; T4.5 and TF0 respectively. The lowest value of mortality was recorded with T1.5 (26.67%) followed by T4.5 (30%); TF0 (30%); T3 (33.33%) and T0 (40%). The reduction of the mortality rate was associated with low but normal value of Red blood cells (RBC), haemoblobin (HGB), haematocrit (HCT) and high but also normal values of mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and lymphocyte percentage (LYM) (Table 4). These values of blood parameters (Table 4) are similar to those of Al-Helali (2012), Saidu *et al.*, (2012) in control group.

Table 4: Effect of dietary supplementation with Feed Ingredient with Probiotics Properties on mortality rate and blood parameter.

	T0	T1.5	T3	T4.5	TF0	P
CMR_W4 (%)	0.27±0.24	0.17±0.20	0.23±0.32	0.10±0.17	0.17±0.08	0.71
CMR_W8 (%)	0.40±0.33	0.27±0.21	0.33±0.30	0.30±0.40	0.30±0.17	0.95
RBC-million/µl	5.78±1.42	4.90±0.56	5.16±0.43	5.84±0.19	4.74±0.45	0.18
HGB (g/dl)	11.08±2.42	9.77±0.62	9.80±0.96	11.10±0.49	9.00±0.69	0.13
HCT (%)	42.85±12.27	35.53±3.09	34.63±3.38	38.45±0.20	33.18±2.03	0.21
MCV (fl)	71.93±0.79	72.8±4.29	67.18±3.48	66±1.80	70.3±5.10	0.06
MCH (pg)	18.75±1.26	20.1±1.84	19±0.67	19.05±0.20	19±0.82	0.48
MCHC (%)	26.2±1.62	27.53±0.93	28.33±1.20	28.85±1.10	27.13±1.11	0.06
WBC (G/L)	5150±1949	8633±3364	6875±2838	5450±367	8325±1445	0.15
NP (%)	5.75±1.26	3.67±0.47	4.75±1.71	5.00±0.82	6.75±3.59	0.27
LYM (%)	91.75±2.06	94.67±1.70	92.75±2.06	93.50±0.41	91.75±3.30	0.29
MONO (%)	2.50±1.00	1.67±1.25	2.50±0.58	1.50±1.23	1.50±1.73	0.58
PLT (G/L)	251.00±160.40	233.70±193.50	152.00±122.90	159.00±37.60	282.30±161.20	0.65

CMR_W4= cumul mortality rate at 4 weeks of fattening; CMR_W8= cumul mortality rate at 8 weeks of fattening; RBC= red blood cells; HGB= hemoglobin; HCT= haematocrit (%); MCH= Mean Corpuscular Hemoglobin (pg); MCHC= Mean Corpuscular Hemoglobin Concentration (%); MCV= Mean Corpuscular volum (fl); WBC= White blood cells (G/L); NP= Neutophils polynuclear; LYM= Lymphocyte percentage; MONO= Monocytes; PLT= Platelet count; Values shown are the mean±SD.

This demonstrates that rabbit fed with FIPP are in normal sanitary condition or have been maintained by FIPP. This reduction of the mortality rate could be explained by the combined effect of the increase in the diversity of bacteria and modifying microbial bacterial activity in the caecum (Bovera *et al.*, 2006, 2012; Abousekken *et al.*, 2015) with the use of FIPP and the production of organic acids from the diet of carbohydrates (acetic acid, propionic acid, butyric acid). Indeed, these fatty acids limit the development of *Escherichia coli* and *Salmonella* as well as the production of hydrogen peroxide and diacetyl by lowering the pH in the intestine (Salminen, 1992; Krehbiel *et al.*, 2003; Grajek *et al.*, 2005). Reid Group Pioneering studies demonstrated that lactobacilli, which are used in manufacturing FIPP, possessed anti-adhesive properties which enabled them to inhibit the adhesion of pathogenic bacteria to the host cells (Reid *et al.*, 1995). In fact, it has been shown that bacteria of the genus *Lactobacillus* produce hydrogen peroxide (H2O2) which has the effect of inhibiting the growth of *Gardenella vaginalis vaginosis* (Mastromarino *et al.*, 2002). Lactobacilli also produce acids such as lactic and acetic acid and hence may lower the pH of very locally and thereby inhibit the growth of pathogens such as *Salmonella enterica*, *Escherichia coli*, etc. (Saltet De Sablet. 2007). The similarity of monocytes values whatever the treatment indicates a quasi-infection similar situation. But in the case of T0 there is a high production of NP to ensure the phagocytosis while in treatments with FIPP is rather that the LYM more. The number of LYM inspires an improvement of the immune system by producing antibodies. In these treatments, the reaction of subjects at FIPP can be explained by the barrier effect or the production of bacteriocins by lactic acid bacteria of FIPP.

Probiotics modulate the immune response of the host by enabling the production of pro and anti-inflammatory cytokines, which allows the recruitment of immune cells to the site of infection (Servin, 2004). Also, for the group of rabbit receiving FIPP, we notice an improvement of growth, sign of good digestion. According to Fortun-Lamothe (2004), nutrients, fruit of digestion, have direct role on immunity as they serve as substrates and enzymes cofactors for cellular multiplication during immune response (phagocytes, lymphocytes) and for synthesis of effective molecules (antibody, complement, nitric oxyde, lysozyme) or informant molecules (cytokines, inflammatory mediators) (Klasing and Leshchinsky, 2000). Furnitures of susbtrates concerned mainly amino acis, fatty acids and glucose while co-factors for enzymatic activities are often vitamins or minerals. Nutrients could also have an indirect effect on immune response by modifying the intra- and extra-cellular communication pathways (cytokines) or limiting undesirable effects of effective molecules. For example, the amount and profile of fatty acids in the diet determines the type of fatty acids which are incorporated into the cell membranes and hence the fluidity of the membranes and the type of eicosanoid secreted as informant molecules. Some anti-oxidants (vitamins E and C, β-carotene) may limit the undesirable effects of destructive molecule (nitric oxide, hydrolases.) on membraneous components (phospolipids). Finally, the immune system is also regulated by numerous hormones (gastrin-releasing peptide, GH, IGF1, insulin, thyroides hormones…) most of which are responsive to nutrionnal factors (glucose, protein/energy ratio…) (Genton and Kudsk, 2003).

The decline of platelets counts in the group fed with FIPP means a fluidity of the blood and modification of viscosity of plasma that can be confer to lactic acid bacteria of FIPP (Tadjine *et al.*, 2008).

Our results are not in agreement with those of Trocino *et al.*, (2005), who reported that rabbit supplemented diet with *Bacillus var* toyi at a rate of 2.10^5 and 1.10^6 spores / g of feed did not reduce the mortality rate but reduced morbidity ranging from 29.12% to 58.24%. The authors further stated that the increase of the dose of *Bacillus var* toyi in the diet was followed by the increasing of mortality and the morbidity.

Feed Cost and Economic Feed Efficiency

After 8 weeks of experiment, feed cost (FC) and Economic feed efficiency (FEI) did not differ significantly ($p<0.05$) between treatment (table 5). FC followed the same trend as the consumption index. The FC was calculated by multiplying food price by the IC.

Table 5: Effect of dietary supplementation with Feed Ingredient with Probiotics Properties on feed cost (FCFA/g live weight) and feed efficiency index (FCFA live weight gain /FCFA of feed).

	T0	T1.5	T3	T4.5	TF0	P
FC_S4	1413.3±180.7	1712.1±603.1	1490±293.1	1602.5±298.0	1323.3±92.0	0.33
FC_S8	2144±913	1860±157	1815±160	2859±2224	1812±268	0.42
FEI_S8	1.29±0.35	1.35±0.12	1.39±0.11	1.15±0.45	1.40±0.19	0.54

FC_S4 = Feed cost at 4 weeks of fattening; FC_S8 = Feed cost at 8 weeks of fattening; FEI = Feed efficiency index at 8 weeks of fattening.

The absolute value of the increase induced by the incorporation of IAPP on food price (5.12%, 10.10% and 14.93% respectively for T1.5, T4.5 and T3) was low compared to that induced on the IC (17.46%, 23.11% and 16% respectively for T1.5, T3 and T4.5). Thus, the lowest FC is also observed with T3 (1815 FCFA / kg gain), followed by TF0 (1812 FCFA / kg gain); T1.5 (1860 FCFA / kg gain); T0 (2144 FCFA / kg gain) and T4.5 (2859 FCFA / kg gain). And beyond 3% of the IAPP incorporation dose in food, the breeder is no longer competitive with his colleagues who do not use the IAPP. According to Daouda *et al.,* (2013), the feed cost has varied between 455 and 954 FCFA / kg gain after 8 weeks fattening. This low value is due to the price of the food that varied from 130 FCFA / kg and 200 CFA / kg against 450 CFAF / kg and 517 FCFA / kg during our experimentation. Houndonougbo *et al.,* (2012) obtained with the same formula food prices varying between 189 CFA / kg and 191 FCFA. According to Montessuy *et al.,* (2009), the rise in food prices related to the increase of energy level in the experimental feeds was compensated by the decrease in consumption (rationing). However, they note that the decline in feed cost is proportional with the increase in energy level. In our case, this variation is related to the increased cost of raw materials.

In general, the FEI show the same trends with feed costs because the average selling price of the product on the market was unique. According to Thoto (2006), the price of live weight of rabbit varies from 1800 to 2500 FCFA/kg for 45% of breeders. But for Kpodekon *et al.,* (2010), this price is 1000 FCFA/kg. But nowadays, the sale price of live weight in kilogram of rabbit is 2500 FCFA. Based on this price, the best value of FEI is 1.39 and was obtained with the T3. The FEI was similar to that of TF0 (1.4) which is the standard treatment. After these treatments, we have the T1.5 (1.35), T0 (1.29) and finally T4.5 (1.15) with the lowest performance. Thus, the dose of 1.5% and especially of 3% gave the best performance. These results corroborate those found by Trocino *et al.,* (2005), where supplementation of the feed of the rabbit, by Bacillus var toyi at a rate of 2.10^5 and 1.10^6 spores / g of feed, improve feed efficiency of 0,1. However, in this study the different kinds of lactic acid bacteria contained in the FIPP are not identified yet. It is only *Lactobacillus* that has been identified in a proportion of 7.63 log CFU / g of food.

Conclusion

The decrease in mortality associated with the FIPP and the increased growth observed in this study suggest that the intestinal microbial population was positively affected by *lactic acid bacteria* in the FIPP. From the analysis of collected data, we can retain that the FIPP, at a dose of 1.5% and 3%, better improves growth performance, feed efficiency and hematologic parameters and reduces mortality.

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