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Effect of Added Quantity of the *Callistemon viminalis* Essential Oil on the *in Vivo* Digestibility of *Pennisetum clandestinum* Hay and Some Biochemical Parameters on the West African Dwarf Goat

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Abstract

The study of the effect of added quantity of the essential oil of *Callistemon viminalis* on the *in vivo* digestibility of *Pennisetum clandestinum* and some biochemical parameters on the West African Dwarf goat was conducted with nine old West African Dwarf goats. After the adaptation period, each animal received 900 and 100 g/day of *Pennisetum clandestinum* hay and concentrate respectively, associated with 0, 100 or 200 mg essential oil/kg of DM. The samples of 100 g of each ration, faeces and 10 ml of urine were collected and analyzed for chemical composition and the evaluation of ingestion and digestibility. Also blood samples were obtained from jugular vein of all goats after *in vivo* digestibility test for the dosage of biochemical parameters. The results of this study show that the ingestion of dry matter, organic matter and the fibers were significantly ($p < 0.05$) higher on the goat with the ration FPc + HECv200. The digestibilities of these same components were equally higher with the ration FPc + HECv200 (71.00% and 69.00% respectively for the dry matter (DM) and organic matter (OM)). Retained (5.64 g/j) and digested (51.33) nitrogen were significantly ($p < 0.05$) higher with the ration FPc + HECv200. The values of blood metabolites studied increased significantly ($p < 0.05$) with added quantity of essential oil in the rations, except for albumin, globulin, glucose and the low-density lipoprotein (LDL). In general, the incorporation of essential oils of *Callistemon viminalis* in the ration improved

ingestion, digestibility and biochemical parameters on the West African Dwarf goat.

Keywords

Hay, Digestibility, *Callistemon viminalis,* Essential Oil, Biochemical Parameters

1. Introduction

Nutrition of ruminants is controlled by the microbial fermentation that occurs in the rumen. This fermentation could be improved in many ways by improving fiber digestion as well as decreasing protein degradation. Modifying protein degradation could increase the efficiency of energy and nitrogen (N) utilization, thus increasing the livestock production [1]. Microbial digestion in the rumen is a major phenomenon whose efficiency can be improved by selective antimicrobial agents such as, antibiotics [2]. Among these additives, ionophores antibiotics such as monensin were employed successfully as food additives for decades to handle ruminal fermentations [2] so as to improve the efficiency of feeding in the system of production of ruminants [3]. However, the risk of the presence of antibiotic residues in milk, meat and its effects on human health led to its prohibition in animal feeding by the European Union. The use of essential oils (EO) in livestock nutrition has been expanded after the ban on the use of antibiotic as growth promoters, and as ionophores [1].

The EO are blends of secondary metabolites that are commonly extracted by steam distillation or solvent extraction [4] [5]. Chemically, they are characterized as having a very diverse composition, nature and activities [6]. The effect of essential oils depends on their structure, which results from the chemical composition and the type of functional group or the aromatic molecules which compose them [7].

Several essential oils and their active components have strong and selective antimicrobial activities against a broad range of microorganisms, including bacteria, protozoa and fungi [8], and can be employed to control the competition between various microbial populations with the objective of improving the efficiency of energy and protein utilization in the rumen [6].

C. viminalis is a shrub of the family of Myrtaceae. It can reach 8 meters height and has hanging branches with leaves of 3 to 7 cm long and 3 to 7 mm wide. The essential oil of *C. viminalis* mainly consists of oxygenated monoterpenes (1,8-cineole, *α*-Terpineol, Terpinen-4-ol…) and hydrocarbon (*α*-Pinene, *β*-Myrcene, *β*-Pinene, α-Terpinolene, *p*-cymene, *γ*-Terpinene…) [9]. These compounds could favorably modulate ruminal fermentations by an increasing the concentration of volatile fatty acid and the quantity of amino acids that are available for the animals' needs. No data are available on the effects of the inclu-

sion of *C. viminalis* essential oil in goat diets, on feed digestibility and rumen metabolism. Research is therefore needed to determine the effects of *C. viminalis* essential oil *in vivo*. The present study is conducted to evaluate the potential of using *C. viminalis* essential oil to improve feed digestibility, ruminal fermentation and some blood metabolites in West African Dwarf goat.

2. Materials and Methods

2.1. Study Area

This study was conducted at the experimental farm and Laboratory of Animal Nutrition of the University of Dschang. The geographic area is within the Sudano-Guinean zone of Central Africa (latitude 5˚ - 7˚N, longitude 8˚ - 12˚E; altitude 1400 m ASL). The annual temperature varies between 16˚C and 27˚C with a relative humidity of 40% - 97%. There are two main seasons: the rainy season (April-October) and the dry season (November-March), which is the main cropping season. The mean of the annual rainfall is about 2000 mm [10].

2.2. Animal Material

Nine adults and empty West African Dwarf goat of average weight 19 ± 2 kg were bought on the market of Dschang. Their ages, determined from their dentition varied from 18 to 24 months. A month prior to the study, all animals were treated with oxytetracycline (20%), a preventive antibiotic. They were also dewormed with Ivermectin 1% (synthetic broad spectrumanthelmintic active against gastrointestinal nematodes and pulmonary adults and larvae).

2.3. Plant Material

Two species of plants (*C. viminalis* and *Pennisetum clandestinum*) were used in this study. The leaves of *C. viminalis* were collected in the campus of the University of Dschang in March 2016 were air dried under shade at ambient temperature. The *C. viminalis* samples chosen for extraction of essential oils were crushed. Fresh leaves of *Pennisetum clandestinum* were cut 45 days after planting at the Experimental Farm of the University of Dschang and then air dried in open shaded air at ambient temperature until chemical composition is presented shown in **Table 1** is obtained. The concentrated food used in this study consisted of: 50% maize, 30% wheat bran and 20% cotton seed cake.

2.4. Extraction of Essential Oil

The leaves of *C. viminalis* were crushed to a powder to enable the extraction of EO which was done in the laboratory of Physiology and Animal Health of the Faculty of Agronomy and Agricultural Science by hydrodistillation according to Wang and Waller [11]. The latter technique consists of placing the *C. viminalis* plant material in an alembic, and heating with water to 200˚C. Intense heat causes the explosion of oil containing saccules of *C. viminalis* to release oil which spreads in the water vapor. Oil water vapor was then channeled in a

Table 1. Chemical composition of *Pennisetum clandestinum.*

Chemical composition	quantity
Dry matter (%)	96
(% ms)	
Ash	15.19
Organic matter	84.80
Total nitrogenized matter	13.37
Lipids	2.09
Crude fibre	30.42
Neutral detergent fiber (NDF)	82
Total carbohydrate	64.82
dMO	32.91

dMO: digestibility of organic matter.

condenser and cooled to be liquified again. At the end, oil was separated from water and was dried using anhydrous sodium sulphate.

2.5. *In Vivo* Digestibility

The nine animals were randomly divided into three groups of comparable weight. They were placed in individual cages with a device enabling separate collection of urine and faeces samples. Each group received one of the following rations:

- Group 1 (control) = 100 g of concentrate + 900 g of *Pennisetum clandestinum* hay + 0 mg essential oil of *Callistemon viminalis* (FPC + HECv0).
- Group 2 = 100 g of concentrate + 900 g of *Pennisetum clandestinum* hay + 100 mg essential oil of *Callistemon viminalis* (FPC + HECv100).
- Group 3 = 100 g of concentrate + 900 g of *Pennisetum clandestinum* hay+ 200 mg essential oil of *Callistemon viminalis* (FPC + HECv200).

The trial period of the experiment lasted 21 days and included 15 for adaptation and 6 days of data collection. The purpose of the adaptation period was to make it possible for animals to familiarize themselves with the digestibility cages and their new ration. During the trial period of the experiment, each animal received 500 g of the experimental ration the first day. This quantity was gradually increased until each animal 100 g of concentrate and 900 g of *P. clandestinum* hay on the last day of adaptation. Water was available at libitum. Essential oil was incorporated in the concentrate before being served to the animals. Ration was distributed twice per day at 0.5 kg at 8 am and at 0.5 kg at 3 pm).

Refused *P. clandestinum* was removed daily and weighed prior to distribution of new ration. Quantity of ingested *P. clandestinum* was determined by substracting the weight of refused quantity from the served quantity. A sample of refused ration was taken to determine the content of the dry matter. Every

morning, faeces produced by each animal were weighed and urines were measured using. the urines produced by each animal were collected in containers inside diluted sulphuric acid (10%) was introduced beforehand according to the average volume of urine produces by each animal during the adaptation period (2.5 ml sulphuric acid for 100 ml urine) to stabilize urinary nitrogen. The volume of the urines thus collected every morning was measured using a 1000 ml graduated test-tube. 10 ml samples of urine were collected in clean bottles and preserved at 4˚C in a refrigerator for later analysis of nitrogen.

A sample of 100 g faeces was collected and dried to a constant weight at 60˚C in a ventilated drying oven, prior to being crushed and preserved for letter chemical analysis. The apparent digestibility coefficient (CUDa) of dry matter (DM), of organic matter (OM) and of nitrogen was calculated respectively using the following formulas:

CUDaDM (%) = (DM ingested − DM excreted) × 100/DM ingested,

CUDaOM (%) = (OM ingested − OM excreted) × 100/OM ingested and

CUDaN (%) = (N ingested − N excreted) × 100/N ingested

2.6. Determination of the Biochemical Parameters

At the end of the trial of *in vivo* digestibility, blood samples were obtained from jugular vein of all goats (by puncture of the jugular vein) and introduced into labelled dry tubes test. Serum samples were collected after blood centrifugation frozen at −20˚C until the day of analyses. They were carried out at the laboratory of Biochemistry of the University of Dschang using the commercial kits (CHRONOLAP S.A.). The experimental protocol which allowed for the determination of the serum concentration of proteinic (total protein, albumin and globuline) and energetics (glucose, triglyceride, total cholesterol, HDL and LDL) parameters in serum samples was described by the manufacturer (CHRONOLAP S.A.). Proportionings were colorimetric and the readings of absorpbance were done using a spectrophotometer.

2.7. Statistical Analysis

Data on ingestion, *in vivo* digestibility and biochemical parameters were subjected to one way analysis of variance (ANOVA) following General Linear Models. When differences exist between treatments, means were separated by the Waller Duncan test at 5% significance level [12].

3. Results

3.1. Ingestion of the Dry Matter (DM), Organic Matter (OM) and Fibers (NDF) of the Various Rations

The incorporation of the essential oil of *C. viminalis* leaves significantly ($p < 0.05$) influenced ingestion of the dry matter, organic matter and fibers of the various rations on the West African Dwarf goat (**Table 2**). Indeed, the ingestion of DM, OM and fibers of the FPc + HECv200 ration were significantly ($p < 0.05$)

Table 2. Ingestion of DM, OM and fibers of the various rations.

Components (%D M)	Rations			SEM	P
	FPc + HECv0	FPc + HECv100	FPc + HECv200		
Dry matter	710.33[b]	651.00[c]	731.67[a]	0.15	0.00
Organic Matter	627.67[b]	575.33[c]	646.67[a]	0.33	0.01
Fiber (NDF)	582.67[b]	533.33[c]	600.33[a]	2.60	0.00

a,b,c: the mean bearing the same letter in the same line are not significantly different ($p > 0.05$) SEM = standard Error of mean; P = Probability.

higher than that of the rations FPc + HECv0 and FPc + HECv100, with the lowest values of these components found with the FPc + HECv100 ration.

3.2. Digestibility of Dry Matter (DM) and Organic Matter (OM) of the Various Rations

The incorporation of the essential oil of *C. viminalis* leaves significantly ($P < 0.05$) improved both the digestibility of the dry and the organic matter of rations in goats (Figure 1). Indeed, the digestibilities of these components significantly ($P < 0.05$) increased with the level of addition of essential oil with the ration. The highest digestibilities of DM and OM were obtained with FPc + HECv200 ration while lowest were obtained with FPc + HECv0 ration.

3.3. Effect of Various Levels of Incorporation of the Essential Oil of *Callistemon viminalis* (HECv) on the Digestive Use of Nitrogen of *Pennisetum clandestinum* Hay

Highest ingestion of nitrogen were observed with the rations FPc + HECv100 and FPc + HECv200, but no significant difference ($p > 0.05$) was observed (Table 3). In the same way, quantities of nitrogen excreted in faeces of the animals receiving rations that contain the essential oil were comparable and significantly weaker than those excreted in faeces of animals receiving ration without essential oil. Moreover, the quantities of urinary nitrogen were comparable ($P > 0.05$) whatever the ration. Quantity of nitrogen retained significantly ($P < 0.05$) increased with the level of incorporation of the essential oil of *Callistemon viminalis* leaves. The quantity of nitrogen retained by these animals fed on ration with 200 mg essential oil was significantly ($P < 0.05$) higher than those of the animals fed on rations FPc + HECv0 and FPc + HECv100. The incorporation of the increasing levels of the essential oil of *Callistemon viminalis* in the ration made it possible to increase ($P < 0.05$) CUDa of nitrogen of *Pennisetum clandestinum* hay in the goat.

3.4. Effect of Various Levels of Incorporation of the Essential Oil of *Callistemon viminalis* on Some Biochemical Parameters

The incorporation of the essential oil of *C. viminalis* leaves in the ration influenced the serum level of some proteinic (total protein, albumin, globulin) and

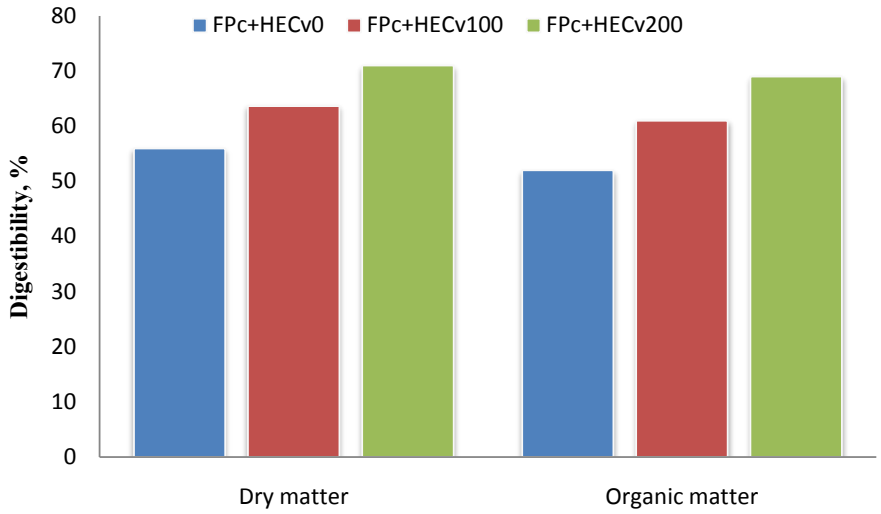

Figure 1. Digestibility of dry matter (DM) and organic matter (OM) of the various rations.

Table 3. Digestive use of nitrogen of the various rations.

Nitrogenized assessment	Rations			SEM	p
	FPc + HECv0	FPc + HECv100	FPc + HECv200		
Nitrogen ingested (g/j)	15.33[a]	14.00[a]	15.65[a]	1.18	0.35
Fecal nitrogen (g/j)	10.33[a]	7.60[b]	7.66[b]	0.19	0.04
Urinary nitrogen (g/j)	3.00[a]	2.37[a]	2.33[a]	0.15	0.21
Nitrogen retained (g/j)	2.33[b]	4.00[a]	5.64[b]	0.15	0.00
Nitrogen Digestibility (%)	32.02[c]	43.00[b]	51.33[a]	0.31	0.00

a,b,c: the mean bearing the same letter in the same line are not significantly different ($p > 0.05$) SEM = standard Error of mean; P = Probability.

energetic (triglyceride, total cholesterol, HDL and of the LDL) parameters in the West African Dwarf goat (Table 4). The incorporation of 200 mg essential oil of *C. viminalis* leaves in the ration made it possible to significantly ($P < 0.05$) increase the quantity of total proteins in the goat. On the other hand, the serum levels of albumin, globulin and glucose in the goat remained comparable ($p > 0.05$) independently of the level of incorporation of essential oil in the ration. It was the same as for LDL level. The incorporation of 200 mg essential oil of *C. viminalis* leaves in the ration contributes to significant increase ($P < 0.05$) of the levels of triglyceride and HDL in the serum of the goat.

Discussion

Ingestions of DM, OM and fibers were significantly improved with incorporation of EO in rations at all levels. These results are in agreements with those obtained by Benchaar *et al.* [13] who showed that the supplementation of 2 - 4 g/day of a mixture of EO in the ration of bovines increased significantly the ingestion of the dry matter. On the other hand, Aouadi and Salem [14] showed

Table 4. Effect of the level of incorporation of the essential oil of *Callistemon viminalis* on some biochemical parameters.

parameters	Characteristics	Rations			SEM	p
		FPc + HECv0	FPc + HECv100	FPc + HECv200		
Proteinic parameters (g/dl)	Total proteins	7.91[a]	6.98[b]	8.02[a]	0.36	0.01
	Albumin	2.64[a]	2.13[a]	2.61[a]	0.34	0.39
	Globulin	5.27[a]	4.84[a]	5.40[a]	0.09	0.10
Energetic parameters (mg/dl)	Glucose	21.00[a]	20.59[a]	22.20[a]	0.22	0.06
	Total cholesterol	47.04[b]	44.32[c]	53.39[a]	0.12	0.00
	Triglycérides	9.13[b]	9.08[b]	12.05[a]	0.09	0.00
	HDL	36.40[b]	33.59[c]	41.73[a]	0.08	0.00
	LDL	8.81[a]	8.91[a]	9.25[a]	0.14	0.49

a,b,c: the mean bearing the same letter in the same line are not significantly different ($p > 0.05$) SEM = standard Error of mean; P = Probability; HDL = highdensity lipoprotein; LDL = lowe density lipoprotein.

that a supplementation by EO of *Rosmarinus officinalis* and *Artemisia herba alba* to an amount of 200 mg/Kg of DM does not affect ($P > 0.05$) the ingestion of food. Similar results were obtained by Santos *et al.* [15]. This variability could be related to the chemical nature of the various EO used by these authors. These effects on ingestion could also be attributed to the flavor and the savor of the EO used [16].

This study found that *in vivo* digestibilities of DM and OM fibers (NDF) in the rations increased with the increasing levels of incorporation of essential oil. Results from this study suggest that supplementing a goat diet with *Callistemon viminalis* EO has the potential to improve the efficiency of feed utilization. This is in disagreement with the results of Khateri *et al.* [17] who observed no change in DM, OM, and crude protein (CP) digestibility, when a sheep EO mixture was added at the dose of 0.8 and 1.6 Ml/d. Castillejos *et al.* [18], also observed no change in DM, OM, and CP digestibility, when a Crina Ruminants EO mixture (a commercial blend of EO) was added at the dose of 3.8 mg/l of ruminal fluid in continuous-culture fermenters. Castillejos *et al.* [19] observed that addition of 5, 50, and 500 mg/l of eugenol in a continuous-culture fermenter did not affect DM digestion. In the same study, thymol at 500 mg/l reduced the digestion of DM, but no effects were observed at lower doses (5 and 50 mg/l). These results suggest that the effects of essential oil components on rumen microbial activity may vary depending on the dose and the type of essential oil component used. Benchaar *et al.* [13] observed that ADF digestibility was significantly increased (3 percentage points) when diets were supplemented with EO 2 g/cow per day. However, ruminal *in sacco* degradation of ADF was not affected by EO addition, suggesting that EO supplementation altered total-tract digestibility by enhancing ADF digestion at post. Discrepancy between studies could be due to experimental conditions (*in vitro* VS *in vivo*, ruminal evaluation VS total tract evaluation), dose of supplementation and kind of EO or it components. In addition, longer

exposure time of ruminal microorganisms (*in vivo* and long time *in vitro* continuous culture vs. short time batch culture study) to EO may allow rumen microbiota to adapt to the EO [20] [21].

The microbial proteins synthesized in the rumen are not sufficient to support the amino acid requirements of high-producing ruminants. Consequently, diets are usually supplemented with sources of feed protein, but such practices can increase feed costs [8]. Furthermore, inefficient nitrogen (N) utilization by ruminants results in excretion of N-rich wastes to the environment. Lapierre *et al.* [22] estimated that about 30% of N consumed by the dairy cow is excreted in urine. Therefore, improving N utilization has a positive impact on efficiency of animal production and on the environment.

McIntosh *et al.* [23] indicated that the anti-microbial action of EO can be exploited to modulate activities of rumen microbial populations by reducing protein degradation and therefore, enhancing N escape from the rumen, and reducing N losses in the urine. Moreover, improved efficiency of N metabolism in the rumen could reduce N losses in feces and urine. More recently, a number of studies have shown that factors such as the chemical composition and dosage level of EO could influence effects of EO on ruminal N metabolism. Further research *in vivo* is therefore needed to assess the effectiveness of EO, to efficiently modify ruminal microbial populations in order to improve protein utilization in ruminants. Results from the present study indicate that the apparent digestibility of the nitrogen (CUDaN) increased significantly with the addition of *Callistemon viminalis* EO with various rations. These results concord with those obtained by Newbold *et al.* [24] and Hristov *et al.* [25]. The effects of EO in the rumen are based on the reduction of degradation of proteins and with an inhibition of degradation of amino acid due to the selective action of their components terpenoïdes on the micro-organisms, specifically the proteolytic and ammonia producing bacteria [26]. What could favorably modulate ruminals fermentations by increasing the quantity of amino acids available for the animal needs could consequently involve an increase in the digestibility of nitrogen. These results corroborate the assertion that addition of essential oil with the ration promotes an increase in the quantity of protein that goes towards the intestine thus improving the efficient use of food proteins. Benchaar *et al.* [27] observed no effect of EO on ammonia concentration and protein degradability in the rumen of lactating cows fed silage-based diets supplemented with EO (1 or 2 g/cow/d). Castillejos *et al.* [18] observed no effect on N degradation when a mixture of EO (Crina Ruminants) was added in continuous culture fermenters. Discrepancies between studies could be attributed to by differences in the procedure used (*in vivo* vs. *in vitro*) and in the duration of exposure of bacteria to EO (24 - 48 h for *in vitro*; 2 - 4 week for *in vivo*), as well as possible adaptation of ruminal bacteria to EO. McEwan *et al.* [28] and Molero *et al.* [29] suggested that the effect of EO on protein degradation is diet and substrate dependent.

Biochemical parameters studied increased with the addition of essential oil in the rations without overflowing the normative values defined by Ndoutamia and

Ganda [30], except the average serum albumin concentration that dropped with the incorporation of the essential oil of *Callistemon viminalis*. The high serum concentration of total protein and globuline which is the proteinic parameters of the metabolism, along with the incorporation of essential oil in the ration can be justified by the effect of terpenoïdes of this oil on the micro-organisms of the rumen, specifically the proteolytic and ammonia producing bacteria which promote a reduction in degradation of the proteins and with an inhibition of the degradation of amino acid that will be used in an efficient way on the level of the intestine [1]. These observations are reinforced by the results obtained with other EO containing the similar components [31]. These results are in disagreement with those obtained by Khateri *et al.* [17] which used a mixture of essential oil with the amounts 0.8 and 1.6 mL/head/d in the ration of the sheep from the alfalfa hay and of the concentrate and did not record any significant difference on the serum metabolites studied. Chaves *et al.* [32] reported that cinnamaldehyde supplementation in lambs did not affect serum's glucose and non-esterified fatty acid concentration, although it did result in a decrease in serum cholesterol concentration. Chaves *et al.* [33] observed that serum's cholesterol and triglycerides were not affected by cinnamaldehyde supplementation at 100, 200, and 400 mg/kg DM. The discrepancy in serum metabolite in response to EO supplementation between the present study with other *in vivo* observation may be attributed to different experimental diet, doses of EO or compounds used and experimental conditions.

4. Conclusion

At the end of this study on the effect of various levels of incorporation of the essential oil of *C. viminalis* on the *in vivo* digestibility of *P. clandestinum* and some biochemical parameters in the West African Dwarf goat, it arises that: the inclusion of the various essential oil levels of *C. viminalis* made it possible to obtain significantly high ingestion of DM, OM, NDF and nitrogen. The incorporation of the essential oil of *C. viminalis* also significantly improved ($p < 0.05$) the digestibility of the DM, OM and nitrogen at all tested level. Biochemical parameters studied were improved with the addition of essential oil in the rations. Although the results of this study are satisfactory with 100 or 200 mg *C. viminalis* essential oil, it would be desirable to deepen this work by evaluating the effect of the addition of 100 or 200 mg of this essential oil on the growth performances in the West African Dwarf goat.

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