BioChemMed

Technium BioChemMed Vol. 5, pp.40-53 (2023)
ISSN: 2734-7990
www.techniumscience.com

The potential of Black Garlic as Livestock Feed Supplement

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Abstract. Black garlic is generated from the fermentation of the fresh ones in one to three weeks at 60-80ºC and relative humidity of 50-99%. This product is derived without additives and is utilized for human or livestock health. In general, it contains bioactive substances, including Antioxidants, Polyphenols, Gallic, Chlorogenic, Caffeic, P-Coumaric Acid, and Flavonoid. These substances can be used as prebiotics and feed supplements for livestock, especially small ruminants. Feed supplements are an alternative solution to promote healthy, economic, and environmentally friendly livestock production, productivity, and performance. Furthermore, healthy livestock implies safe and healthy food production for humans.

Keywords: Potency, Black Garlic, Fermentation, Feed Supplement, Livestock

INTRODUCTION

Livestock productivity is strongly influenced by adequate feeds, rearing management, and health (performance). Over the years, feed additives like antibiotic growth promoters (AGP) have been used for efficient livestock feed production, causing problems that require a solution. Furthermore, AGP used in livestock production including meat and milk is banned because of its residue (Kamra *et al*., 2012). There are continuous efforts in improving nutrition content in feeds for productivity and efficiency, including supplementing with natural ingredients (herbal). This includes black garlic, which can be used as a basic ingredient of feed supplements.

Garlic (*Allium sativum* Linn) is an aromatic herbal plant used as cooking spices, and as traditional medicine for various diseases (Bae *et al.*, 2014; Calle *et al.*, 2017; Ríos *et al.*, 2019; Batiha *et al.*, 2020), and nutraceutical properties in the past (Atif *et al.*, 2021). Furthermore, it contains various nutrients, such as protein (6.3 g), amino acid, carbohydrate (29.8 g), fiber, lipid, pectin, mucous compound, vitamin A, vitamin B1, vitamin B2, essential oils (0,1%) (Sandrakirana *et al.*, 2020; Najman *et al.*, 2021), and polyamine (Atif *et al*., 2021). Garlic contains minerals, such as calcium (103.91 mg/100 g), sulfur (1.138 mg/100 g), natrium (246.34 mg/100 g), magnesium (276.47 mg/100 g), iron, zinc, selenium, phosphorus (310 mg), chlorine, iodine, nickel, cobalt, and chromium (Kang, 2016; Sandrakirana *et al.*, 2020; Atif *et al.*, 2020; Najman *et al.*, 2021). Additionally, it contains antioxidants, anti-inflammation anti-microbial, anti-cancer anti-hepatopathy, neurotropic, and various healthy bioactive compounds (Bae *et al.*, 2014; Najman *et al.*, 2021). Researchers are attracted to the potency of garlic, investigating through intensive discussions (Bae *et al.*, 2014; Lencha and Buke, 2017).

Garlic uses are increasingly popular, especially for medical purposes including antibacterial, antiinflammation, antibiotic, anticancer, antitumor (Bharat *et al.*, 2014; Leontiev *et al.*, 2018; Chen *et al.*, 2019; Sawitri, 2005), anti-Alzheimer (Batiha *et al.*, 2020), antifatigue, regulating blood glucose and pressure, promoting digestion, and increasing appetite (Lu *et al.*, 2017). Garlic is processed into various products through technology, such as oil, powder, and black garlic, currently popular for health. Consumers and scientists have increased the demand for these products in recent years because of their health benefits, considered the healthiest food or "superfood" (Najman *et al*., 2020).

The fermentation involves a thermal process with enzymatic and non-enzymatic browning reactions, such as Maillard, oxidation, and caramelization, radically changing the physicochemical, organoleptic, sensory, and bioactive properties (Najman *et al.*, 2020). Thermal processing significantly increases the biological, pharmacological, and functional components' effects, such as reducing sugar, polyphenol, organic acid, and β-carboline alkaloids, hence strengthening the efficacy of black garlic over fresh one (Lu *et al*., 2018); Qiu *et al*., 2018). The physicochemical properties changes increase the bioactivity of black garlic (Kimura *et al*., 2017). Therefore, the potential of single black garlic bioactive compound should be researched as the basic ingredient of herbal livestock feed supplements.

THE CHARACTERISTIC OF SINGLE GARLIC

Single garlic (*Allium sativum* var. solo garlic) has one bulb (Januarti *et al.*, 2019; Susanti *et al.*, 2020), originating from Yunan Province in China (Neeraj *et al*., 2014), owned by the Alliaceae family (An *et al*., 2022), with various names, such as Bawang Lanang as known in Java, Solo Garlic, Single Clove (Gofur *et al*., 2019), Single-Bulb (Bharat *et al*., 2014), Pearl, and Monobulb (European Commission, 2010). Based on its tuber, garlic is divided into single- and multi-bulb. The general characteristics of both include a pungent smell and taste from their organosulfur compounds (Ríos *et al*., 2021). However, a single bulb has a stronger and pungent taste and aroma due to chemical contents like allicin (Bharat *et al*., 2014).

Single garlic as shown in Figure 2 contains 5-6 times higher bioactive compounds and hepatoprotective effects than a multi-bulb as illustrated in Figure 1 (Tran *et al.*, 2018; Susanti *et al.*, 2020). Fresh multi-bulb contains moisture content 62-68%, carbohydrates 26-30%, protein 1.5-2.1%, amino acid 1-1.5%, fiber 1.5%, non-sulfur including steroid glycoside, essential oil, polyphenol, vitamin B1, vitamin B2, vitamin B6, vitamin C, and vitamin E of 1.1-3.5%, fat 0.5% (Lu *et al.*, 2018;

Ríos *et al.*, 2019), and *S-allyl Cysteine* 20-30µg/g (Bae *et al.*, 2014). In contrast, a single bulb contains antioxidant 212.169 ppm of total phenolic 238.558 mg GAE/gr, and total flavonoid 656,41 mg QE/gr (Susanti *et al.*, 2020).

Figure 1. Multi-bulb garlic (Bharat *et al.*, 2014).

Figure 2. Single-bulb garlic (Photo document: Mayulu, 2021).

Fresh garlic contains fructans and a small amount of reducing sugar (Lu *et al*., 2018). Fructans are the polysaccharide component (Yuan *et al*., 2018) that should be considered when evaluating the quality of garlic and its products (Lu *et al*., 2018). Furthermore, they belong to the 1-ketose family of fructose and glucose in a 14:1 ratio (Yuan *et al.*, 2017) and are unstable in high-temperature and acidic environments (Lu *et al.*, 2018).

Single garlic contains inulin (Wikandari *et al*., 2020) and strong antioxidants such as flavonoids that lower blood sugar, prevent cell damage, restore insulin receptor sensitivity, and increase insulin sensitivity (Batiha *et al*., 2020; Susanti *et al*., 2020). The flavonoids reduce the production of Reactive Oxidative Species (ROS). These (ROS) are free radicals in the body that oxidizes fat, protein, and nucleic acid, to damage biomembranes and muscle structures that cause muscle damage and fatigue (Januarti *et al*., 2020; Najman *et al*., 2021).

Research showed that single garlic has an IC_{50} value of 10.61 mg/ml (Prasonto *et al.*, 2017). This indicates that the antioxidant content is stronger and different from multi-bulb, with an IC_{50} value of 13.61 mg/ml (Prasonto *et al.*, 2017). The IC_{50} value is influenced by the secondary metabolite content, namely phenolic (Januarti *et al*., 2019). Furthermore, these phenolic compounds act as protons, neutralize free radicals (Susanti *et al*., 2020), and transfer electrons into radical compounds (Januarti *et al*., 2019).

Other bioactive compounds in single garlic include Allicin, Ajoene, Diallyl Sulfide (DAS), Diallyl Disulfide (DADS), and Diallyl Trisulfide (DATS) (Gofur *et al.*, 2019). Allicin and Ajoene contain antibacterial properties that inhibit the synthesis of bacterial RNA, DNA, and protein. This mechanism inhibits the work and growth of bacteria, thereby killing their cells (Gofur *et al*., 2019). The pungent flavor and antibacterial activity in garlic depend on allicin produced by the alliin lyase enzyme (Bharat *et al*., 2014). Furthermore, allicin is an important compound in plants (Bharat *et al*., 2014).

Allicin in single garlic essential oil degrades bacterial cell walls by weakening the peptidoglycan layer and modifying cell membranes, shrinking, swelling, and damaging the cells. The cell membrane damage decreases permeability (Gofur *et al*., 2019). Additionally, allicin shrinks bacterial cells and inhibits their metabolism by reducing ATP for cell growth, killing cells (Gofur *et al*., 2019). Diallyl Sulfide (DAS), Disulfide (DADS), and Trisulfide (DATS) in single garlic contain hydrophobic properties that damage phospholipids in the bacterial membrane and causing penetration of cell membranes into the cytoplasm and other cells, killing pathogenic bacteria (Gofur *et al*., 2019). The phytochemicals and phytomolecules content, including organosulfur compounds, phenolic, flavonoid, and vitamins, indicate the potential of single garlic in supporting health (Januarti *et al*., 2019).

Garlic has an antimicrobial effect and a prebiotic function (stimulator for microbes) that improves the microbial ecosystem in the intestine (Kamra *et al*., 2012). Inulin content is a strong prebiotic and cannot be degraded in the gastrointestinal tract, hence can be utilized by the microflora in the large intestine. The results showed that inulin administration increases hydrogen production without methane emission but increases butyrate concentration four times higher than fibrous feed (Kamra *et al*., 2012). Adding at least 1% of dry matter in garlic for sheep concentrate with a ratio of 1:1 reduces methane emission by 11% (l/kg DDMI) (Kamra *et al*., 2012).

BLACK GARLIC PROCESSING TECHNOLOGY

Garlic is used as a cooking spice and an ingredient in medicine and health supplement, although its utilization is limited. The slightly spicy taste and pungent flavor limit consumption and utilization rate (Ríos *et al.,* 2021), hence various processing methods are continuously developed as a solution. The garlic processing technology is diverse, including heat treatment, aging, thermal fermentation without microbes (Bae *et al*., 2014), and steam distillation (An *et al.*, 2022). Processing increases the phytochemical content, reduces the pungent taste and flavor, and enhances palatability (Bae *et al*., 2014).

Figure 3. Multi-bulb black garlic (Choi *et al.*, 2014).

Figure 4. Single-bulb black garlic (Documentation of Mayulu, 2021).

Black garlic is derived through fermentation for one to three weeks at 60-80°C and controlled humidity of 50-99% without additives (Calle *et al.*, 2017; Ríos *et al.*, 2021). This is achieved by heating fresh garlic bulbs (Bae *et al*., 2014), inhibiting bacteria growth, especially Lactobacillus, in black garlic incubation (Thalia *et al.*, 2020). Furthermore, the process excludes microorganisms of bacteria and fungi and preservatives, depending on enzymes from biochemical changes reaction (Najman *et al*., 2020).

The quality of black garlic is affected by temperature, humidity, and fermentation duration (Kimura *et al*., 2017). The garlic's moisture content regularly decreases during heating to produce black garlic (Bae *et al.*, 2014). Furthermore, the processing method and cultivar affect the quality of black garlic’s bioactive value (Setiyoningrum *et al.*, 2018). The thermal fermentation method is widely applied as it changes the physicochemical properties of garlic, including taste, color, texture, and nutrition content (Bae *et al.*, 2014). This treatment induces non-enzymatic browning reactions, such as Maillard, caramelization, and the chemical oxidation of phenol (Bae *et al*., 2014; Lu *et al*., 2018). The increased temperatures during fermentation increase the intensity of the production of Maillard reaction products (Najman *et al*., 2020). The non-enzymatic browning reaction forms strong antioxidant compounds (Bae *et al*., 2014).

Thermal processing changes the physical and chemical contents and forms a biological compound in fresh garlic (Lu *et al*., 2018). Fresh garlic contains an unstable organosulfur compound, converted into stable and soluble substances through fermentation. This compound is the *S-allyl- L-cysteine* with high antioxidant activity (Najman *et al*., 2020). Furthermore, increased antioxidant capacity is from increased polyphenol and S-allyl-cysteine, derived from alliin during thermal processing (Medina *et al*., 2019). The thermal process involves the degradation of polysaccharides, monosaccharides, and oligosaccharides. Polysaccharides decrease gradually from 98.4% to 29.4%, while monosaccharides and oligosaccharides increase from 1.6% to 70.6% (Lu *et al*., 2018).

Thermal processing turns garlic black with a chewy and elastic consistency, weak aroma, not as sharp as garlic, slightly sour and sweet taste (Bae *et al*., 2014; Lu *et al*., 2018; Ríos *et al*., 2021), and soft texture (Qiu *et al*., 2018) and slightly bitter taste like coffee (Qiu *et al*., 2021). The intensity of the brown color in the black garlic increases in higher temperatures during processing (Bae *et al*., 2014). Furthermore, the degree of browning is determined through color analysis using a calorimeter (Lu *et al*., 2018). The sensory evaluation obtained good quality and black color with the ideal temperature of 70-80°C, while 60°C was not recommended as it produced an imperfect black color (Kimura *et al*., 2017).

The slightly sour garlic taste is caused by the pH changes during the Maillard reaction and organic acid formation. The pH decrease occurs in increased processing time and acetic acid from hexose degradation during thermal processing (Yuan *et al*., 2018). Changes in color from white to black and taste are caused by an enzymatic and non-enzymatic chemical reaction, namely Maillard, between glucose, fructose, and amino acid (Yuan *et al*., 2018; Qiu *et al*., 2021) (Calle *et al*., 2017). The Maillard reaction is essential in making black garlic because it greatly affects the color and taste (Yuan *et al.*, 2018). Furthermore, this reaction produces melanoidin, giving a unique flavor and appearance of chocolate color (Zhao *et al*., 2021). Melanoidin causes a slightly bitter taste in black garlic (Yuan *et al*., 2018), while the color change from white to black reflects the degradation of polysaccharides (Lu *et al*., 2018).

A sweet taste and distinctive aroma are caused by the formation of aldehyde, the dominant compound in garlic (Ríos *et al*., 2021) and a high reduction of sugar content up to 80% (Lu *et al.*, 2018). The sugar content reduction in the Maillard reaction reached 214.9 mg/g (Lu *et al*., 2017). Furthermore, increased fructose level sweetens the black garlic (Yuan *et al*., 2018). Other changes include degradation of polysaccharides (Lu *et al*., 2018) and fructans into monomer, increased *5-hydroxymethyl-2-furaldehyde* up to 6 (six) times more than fresh garlic, polyphenol, and flavonoids (Ríos *et al*., 2021).

Temperature and garlic compounds are important factors that affect the antioxidant activity during processing (Bae *et al.*, 2014). The antioxidant content of black garlic is higher than unprocessed ones (Bae *et al*., 2014). Research showed that the antioxidant activity of black garlic inhibits DPPH radicals by 44.77% (Thalia *et al*., 2020). The organoleptic properties of black garlic increase from the compounds change into stable and flavorless, such as *S-allyl-L-cysteine* (SAC), or decomposition into organosulfur such as *diallyl sulfide (DAS), diallyl disulfide (DADS), diallyl trisulphide (DATS), dithiins,* and *ajoene* (Medina *et al.*, 2019).

Table 1. Bioactive Compounds of Garlic Based on Conventional and Organic Cultivation

Bioactive compound	Organic Garlic	Organic Black Garlic	Conventional Garlic	Conventional Black Garlic
Antioxidant Activity µmol TEAC/g d.m	363.42	654.93	273.65	546.26
Total Polyphenol Content mg GAE/g d.m	7.28	15.10	5.62	12.50
Total Phenolic Acids mg/100 g d.m	49.91	75.50	38.67	54.97
Gallic acid mg/100 g d.m	10.82	14.42	9.54	11.49
Chlorogenic acid mg/100 g d.m	5.25	21.44	5.66	14.88
Caffeic acid mg/100 g d.m	7.85	14.42	4.19	6.48
P-coumaric Acid mg/100 g d.m	25.36	24.69	18.71	21.59
Ferulic Acid mg/100 g d.m	0.64	0.53	0.57	0.54
Total Flavonoids mg/100 g d.m	49.29	63.32	35.02	53.53
Catechin mg/100 g d.m	17.42	8.11	9.54	9.74
Epicatechin mg/100 g d.m	3.73	3.94	3.17	3.29

Gallate Epigallocatechin mg/100 g d.m	6.38	2.94	4.46	3.72
Myricetin mg/100 g d.m	3.37	3.43	3.13	3.46
Quercetin mg/100 g d.m	10.89	34.39	11.11	25.06
Kaempferol mg/100 g d.m	7.50	10.51	3.62	7.26

Source: Najman *et al.*, 2020.

Table 2. Comparison of Phytochemical Properties of Fresh and Black Garlic

Phytochemical Properties	Initial Concentration	Comparison of Phytochemical Properties of Fresh and Black Garlic
Water-soluble sugar	450 mg/g	Increase 1.88-7.91 fold
Polyphenol	13.91 mg/GAE/g	Increase 4.19 fold
Flavonoid	3.22 mg RE/g	Increase 4.77 fold
Amadori & Heyns	10 μg/g	Increase 40-100 fold
Fructans	580 mg/g	Decrease 0.15-0.01 fold
Leucine	58.62 mg/100 g	Increase 1.06 fold
Isoleucine	50.04 mg/100 g	Increase 1.67 fold
Cysteine	81.06 mg/100 g	Decrease 0.58 fold
Phenylalanine	55.64 mg/100 g	Increase 2.43 fold
Tyrosine	449.95 mg/100 g	Decrease 0.18 fold

GAE = Gallic Acid Equivalents; RE = Rutin Equivalents
Source: Kimura *et al.*, 2017.

Table 3. Physicochemical Properties of Black Garlic

Processing Condition	Physicochemical Properties						
	Humidity (%)	Crude protein (g/100g)	Crude Fat (g/100g)	pH	Brix	Mineral Amount (g/100g)	Color
40-90ºC, 11 days	58.48	0.98	0.56	4.22	_	0.10	L:23.70 a:5.90 b:5.58
70ºC, 90% RH, 35 days	29,88	_	_	3.74	_	_	L:4.33 a:2.73 b:3.86
75ºC, 85% RH, 8 days	31.28	14.3	_	_	_	_	E:60
72ºC, 90% RH, 33 days	0.91	_	_	3.49	49.7	_	L:18.01
75 ºC, 90% RH, 21 days	0.94	_	_	3.53	45.3	_	L:18.04
78ºC, 90% RH, 14 days	0.93	_	_	3.80	44.0	_	L:19.06
70ºC, 90% RH, 21 days	31.12	0.97	_	4.22	_	_	L:5.61 a:5.23 b:3.07

RH= Relative Humidity
Source: Ríos *et al.*, 2019.

Table 4. Organosulfur Compounds in Black Garlic Based on Processing Condition

Processing Condition			Organosulfur Compounds
60ºC	80% Relative Humidity	69 Days	~0.2 Allicin/kg
70ºC		33 Days	~0.2 Allicin/kg
80ºC		24 Days	~0.2 Allicin/kg
90ºC		12 Days	~0.2 Allicin/kg
40ºC	70% Relative Humidity	45 Days	124.67µg SAC/g DM
55ºC			113.25 µg SAC/g DM
70ºC			113.25 µg SAC/g DM
85ºC			85.46 µg SAC/g DM
70ºC	90% Relative Humidity	20 Days	746 ppm SAC
		15 Days	1140 ppm SAC
		10 Days	952 ppm SAC
65-80ºC	70-80% Relative Humidity	30-40 Days	194 µg SAC/g
40-90ºC		11 Days	0.39 Thiosulfate
75ºC	85% Relative Humidity	8 Days	0.24 mg/ml Thiosulfinate

DM=Dry Matter; SAC: S-allyl cysteine
Source: Ríos *et al.*, 2019.

THE POTENCY OF BLACK GARLIC FOR HUMANS

Garlic is used as a natural flavoring ingredient in food, traditional medicine, and health supplement. Literature mentioned its health benefits, including Lu *et al*. (2017) which analyzing the effect of black garlic in the prevention of atherosclerosis by cleaning cholesterol and increasing hyperlipidemia using. Further research found that black garlic and extracts can prevent and treat diabetes mellitus, cardiovascular disorders, anticancer, anti-inflammatory, hypoglycemic, atherosclerotic, hyperlipidemia, thrombosis, hypertension, dementia, infections, metabolic disorders (Bharat *et al*., 2014), decreased gastric acid (Calle *et al.*, 2017), antitumor (Draghichi and Luchian, 2015), hypoallergenic, and hypolipidemic (Lu *et al*., 2018). Furthermore, it possesses antiobesity, hepatoprotective, and immunomodulatory activities (Kimura *et al*., 2017).

Antioxidants such as 5-hydroxymethylfurfural (5-HMF) in black garlic fight free radicals more than fresh ones (Calle *et al*., 2017). Black garlic contains S-Allyl-l-Cysteine Sulfoxide (alline) five to six times higher than in fresh one (Bae *et al.,* 2014; Choi *et al.,* 2014; Najman *et al.,* 2020). Allicin is a bioactive compound (Bharat *et al*., 2014) that lowers blood pressure (Rahmayanti and Hanriko, 2020). The results showed that black garlic increases insulin resistance, High-Density Lipoprotein (HDL)-

cholesterol levels and decreases serum cholesterol and triglycerides (Rahmayanti and Hanriko, 2020). Furthermore, research by Gofur *et al*. (2019) reported that the essential oil in single garlic has antioxidants, hence can treat bacterial skin infections by inhibiting the growth of *P. aeruginosa* bacteria using the concentration of 100 mg/ml. The high antioxidant content of black garlic effectively prevents metabolic diseases and alcoholic hepatotoxicity (Bae *et al*., 2014).

THE POTENCY OF BLACK GARLIC FOR LIVESTOCK

Garlic has phytochemical compounds beneficial for livestock (Nelwida *et al*., 2019). Besides its antimicrobial function, it can be used as a prebiotic (stimulator for microbes) to improve the microbial ecosystem in the intestine. Inulin content is a strong prebiotic that cannot be degraded in the gastrointestinal tract, hence can be utilized by the microflora in the large intestine (Kamra *et al*., 2012). The results showed that using black garlic flour in broiler rations as much as 5% could be tolerated without disturbing palatability and increasing broiler breast meat's relative weight to 25.83-27.86%. This is caused by the effective absorption of nutrients. Allicin eliminates pathogenic bacteria and parasites in the digestive tract to properly digest and absorb the ration. The black garlic compounds prevent gram-positive and negative bacteria development and growth by inhibiting RNA production and lipid synthesis.

Alliin compounds contain witch rays that function as mitogenic radiation. Gurwitch rays accelerate and multiply body cells to optimize the broiler body weight. Furthermore, the fat mass of broiler meat given by 5% black garlic flour in the ration decreases. This is due to the Allicin and sulfur compounds (scoring) that bind endogenous bile salts, forming fat emulsion in micelles form inhibited and decreasing absorption. Allicin inhibits the enzyme's function in fat synthesis, affecting the fat metabolism process (Berliana *et al*., 2020).

Quail (*Coturnix-Coturnix japonica*) consumed rations with 3% black garlic flour, resulting in body weight at the first laying of 127.50-131.75 g/head and 39-41 days of first laying age (Berliana *et al*., 2018). The results showed that black garlic flour reduces cholesterol, triglycerides, and Low-Density Lipoprotein (LDL) in laying quail. Additionally, administering black garlic flour by 3% reduced quail egg yolk fat (Berliana e*t al*., 2020).

CONCLUSION

Black garlic is derived from fresh garlic used for human and livestock health. The various bioactive compounds can be used as prebiotics and herbal for livestock feed supplements. Therefore, it can be tested and researched further as ruminant commodities, especially the young ones (goats and sheep). Black garlic-based feed supplements are an alternative solution in the production, productivity, and performance of healthy livestock, economical and environmentally friendly. Healthy livestock impacts food products that are safe and healthy for humans.

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Data Availability

The datasets supporting the conclusions of this research are available from the corresponding author upon request.

Authors' Contribution

Conceptualization-H.M.; Writing-Review and Editing-E.S.; Formal Analysis-H.M., E.S.; Investigation-H.M.; Data Curation-H.M.; Methodology-E.S.; Visualization-H.M.; Resources-H.M., E.S.; Supervision-H.M., E.S.; Writing-Original Draft-H.M., E.S.; Project Administration-H.M., E.S.

Declaration of Conflicting Interests

The authors declare no conflict of interest.

Acknowledgments

Acknowledgments are conveyed to the journal articles (research and reviews) used as references to write a review article titled "Black Garlic as Livestock Feed Supplement."

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