

合欢树皮水提取物替代肉鸡日粮中的抗生素饲料添加剂血液学、血清指数和氧化状态

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摘要: 对375只1日龄肉鸡(Ross 308)的混合性别进行了血液学、血清生化指标和氧化状态的研究,探讨了合欢树皮水提取物作为抗生素饲料添加剂替代物在肉鸡日粮中的作用。在完全随机设计中,鸟类被分为五组,每组十五(15)只,每组五个重复。治疗1(基础饮食+0%ATSM)、治疗2(基础饮食+1.2克土霉素/升水)、治疗3(基础饮食+每升水10毫升ATSM)、治疗4(基础饮食+20毫升ATSM每升水)和治疗5(基础饮食+30毫升ATSM/升水),试验持续56天。一些血液学参数的结果显示,红细胞(RBC)、包细胞体积(PCV)、血红蛋白(Hb)、平均红细胞体积(MCV)、平均血红蛋白(MCH)、平均血球血红蛋白浓度(MCHC)、白细胞(WBC)及其差异在治疗组间有显著性差异($P<0.05$)。ATSM对总蛋白、葡萄糖、尿素、胆固醇、肌酐、天冬氨酸氨基转氨酶(AST)和丙氨酸氨基转移酶(ALT)有显著影响($P<0.05$)。ATSM对超氧化物歧化酶(SDA)、谷胱甘肽过氧化物酶(GPx)、过氧化氢酶(CAT)和丙二醛(MLA)活性有显著影响($P<0.05$)。结论是,肉鸡可以以30毫升/升的剂量服用ATSM,而不会对鸟类的一般性能产生任何负面影响。

关键词: 合欢; 肉鸡; 血液学; 血清生化指标

Albizia lebbeck Stem Bark Aqueous Extract as Alternative to Antibiotic Feed Additives in Broiler Chicks Diets: Haematology, Serum Indices and Oxidative Status

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Abstract: A total of Three hundred and seventy five (375) one day old (Ross 308) broiler chicks with mixed sex were used to examine the effects of Albizia lebbeck stem bark (ATSM) aqueous extract as alternative to antibiotic feed additives in broiler chicks diets: haematology, serum biochemical indices and oxidative status. Birds were divided to five treatments with five replicates of fifteen (15) birds in a completely randomized design. Treatment 1 (basal diet + 0 % ATSM), treatment 2 (basal diet +1.2 grams Oxytetracycline per litre of water), treatment 3 (basal diet + 10 ml ATSM per liter of water), treatment 4 (basal diet + 20 ml ATSM per litre of water) and treatment 5 (basal diet + 30 ml ATSM per liter of water) and the trial lasted for 56

days. Results on some haematological parameters revealed that red blood cell (RBC), pack cell volume (PCV), haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cell (WBC) and its differentials were significantly ($P<0.05$) different among the treatments. Total protein, glucose, urea, cholesterol, creatinine, aspartate aminotransaminase (AST) and alanine aminotransferase (ALT) were significantly ($P<0.05$) affected by ATSM. Activities of superoxide dismutase (SDA), glutathione peroxidase (GPx), catalase (CAT) and malonyldialdehyde (MLA) were significantly influenced by ATSM ($P<0.05$). It was concluded that ATSM could be administered to broiler chicks at 30 ml/litre without any negative effect on the general performance of birds.

Keywords: Albizia lebbeck; Broiler chicks; Haematology; Serum biochemical indices

1 简介

植物学是由植物产生的饲料添加剂的异质组分，由草本植物、水果、香料和其他植物部分组成 (Santi 和 Kim, 2017)。根据 Veerschari 等人 (2011) 的研究，全球有超过 10 万种植物被用于医疗目的，其中许多植物被资源贫乏的小农用来治疗牲畜的各种疾病 (Mirzaei Aghsaghali, 2012; Alagbe 等人, 2020)。大多数药用植物富含矿物质、维生素、氨基酸和生物活性化学物质 [植物化学物质] (Olafadehan 等人, 2020)。然而，对其药理特性的探索或研究只占很小的比例 (世界卫生组织, 1992)。植物中的营养素对动物对疾病挑战的反应有很大影响，并且与免疫系统有直接关系 (Gary 和 Richard, 2002)。合欢是众多用于治疗目的的植物之一。

合欢 (含羞草科) 是一种多年生树木，原产于亚洲和非洲的热带和亚热带地区。合欢属由近 150 种物种组成，遍布印度、中国、尼日利亚、塞内加尔、加纳、多哥、刚果、贝宁、安哥拉、乌干达、博茨瓦纳等地 (Ukpabi 和 Offor, 2018; Karuppannan, 2013)。植物部分 (茎、叶和种子) 被发现含有矿物质 (钙、磷、铁、铜、锌、硒、钼和钾)、维生素和氨基酸 (Alagbe 和 Soares, 2018; Uzoekwe 和 Mohammed, 2015; Mohammed 等人, 2012)。叶和茎传统上用于治疗发烧、牙痛、伤口、麻风病、溃疡、感冒、麻风、性传播疾病和其他呼吸道感染 (Labaran, 2016; Uwaya 等人, 2017)。

关于合欢生物活性的几份报告显示，合欢具有抗菌作用 (Labaran 等人, 2016)、抗炎作用 (Gupta 等人, 2004)、抗氧化剂 (Mc Donald 等人, 2001)、止痛剂 (Sharma 等人, 2007)、抗蠕虫作用 (Karuppannan, 2013)、肝保护作用 (Edeoga 等人, 2005; Alagbe, 2019)、抗糖尿病作用 (Karuru 等人, 2007)、，由于存在生物碱、黄酮、皂苷、酚类、鞣质等多种生物活性化学物质，免疫调节 (Sharma 等人, 2007) 和抗高脂血症特性 (Ueda 等人, 2003) (Labaran 等人, 2016)。鉴于这些丰富的潜力，向鸟类施用合欢树干树皮可能会在遇到挑

战时提供营养以满足所有身体需求。

因此，本实验旨在确定合欢树皮水提取物作为抗生素饲料添加剂替代品在肉鸡日粮中的作用：血液学、血清生化指标和氧化状态。

2 材料和方法

2.1 研究区域

该实验于 2019 年 4 月至 6 月在印度古吉拉特邦苏米特拉研究所动物营养司进行。

2.2 合欢树皮提取物的来源、收集和制备

合欢属植物的茎来自印度古吉拉特邦的不同植物，并经植物学家 Sharma Xing 鉴定。将树皮切成小块，用蒸馏水彻底清洗，在阴凉处风干，以保持试验材料中的生物活性化学物质。使用杵和臼将干燥的样品粉碎成粉末，然后将 250 克样品浸泡在 1000 升水中，连续搅拌样品，并将其保存在 4°C 冰箱中 72 小时。使用沃特曼滤纸过滤所有混合物，并将过滤器 (ATSM) 收集到一个干净的贴有标签的容器中。

2.3 实验动物及管理

实验选用 375 只 1 日龄 (罗斯 308) 混合性别肉鸡。这些鸟从印度的一家商业孵化场购买，抵达农场后称重，以获得初始体重，随后每周称重一次。使用了深垃圾房系统，在研究开始前两周对其进行熏蒸，并每天清洁周围环境，以确保适当的卫生。在完全随机设计中，鸟类被分为五组，每组十五 (15) 只，每组五个重复。使用了电动孵化器，木屑用作垃圾材料。每日饲料摄入量 (g) 计算为提供的饲料和剩余饲料之间的差值。根据环境中的流行疾病情况接种疫苗，并在持续 56 天的整个实验过程中严格遵守所有其他管理实践。

2.4 配料配方

根据 NRC (1994)，在不同生产阶段配制了三 (3) 种基础日粮，以满足鸟类的需求。肉鸡初熟泥 (0–21 天)、种植者泥 (22–35 天) 和修整者泥 (36–56 天)。

治疗 1 (基础饮食 +0%ATSM)、治疗 2 (基础饮食 +1.2 克土霉素 / 升水)、治疗 3 (基础饮食 + 每升水 10 毫升

ATSM), 治疗4(基础饮食+20毫升ATSM每升水)和治疗5(基础饮食+30毫升ATSM/升水)。

2.5 测量参数

采用AOAC(2000)的官方分析方法测定了实验饮食的近似成分。氨基酸分析采用印度NH-09b型离子交换色谱法, 使用氨基酸分析仪进行。

2.6 血液学和血清生化分析

早上很早就从三(3)只随机选择的禽鸟的翅膀静脉采集血液样本, 每重复一次, 用23号针头将血液样本注入5 ml无菌注射器中, 然后转移到乙二胺四乙酸(EDTA)瓶中。血液学参数: 使用自动机器(印度KU-30 HG型Sysmex)分析包细胞体积(PCV)、红细胞(RBC)、血红蛋白(Hb)、平均红细胞血红蛋白(MCH)、平均血红蛋白浓度(MCHC)、平均细胞体积(MCV)、白细胞(WBC)及其差异。

使用不含EDTA的瓶子进行血清分析, 使用由默克印度有限公司(PS-09R型)制造的诊断试剂盒(Olubukola等人(2015)所述)分析血液中的总蛋白、白蛋白、球蛋白、葡萄糖、胆固醇、肌酐、丙氨酸转氨酶(ALT)和天冬氨酸转氨酶。

2.7 抗氧化剂状态

超氧化物歧化酶(SDA)、谷胱甘肽过氧化物酶(GPx)、过氧化氢酶(CAT)和丙二醛(MLA)的活性采用Mahipal等人(2015)概述的方法进行测定。

2.8 统计分析

所有数据均采用SPSS(18.0)进行单向方差分析(ANOVA), 并用Duncan多区间检验(Duncan, 1955)分离显著性均值。P≤0.05为显著。

表1 实验日粮的化学成分

Materials	Starter (1-21 days)	Grower (22-35 days)	Finisher (36-56 days)
Maize	50.00	56.00	60.50
Wheat offal	8.00	7.00	8.05
Soya meal	28.55	22.00	21.00
Groundnut cake	10.00	11.55	6.05
Fish meal	2.00	2.00	2.00
Bone meal	0.35	0.40	0.40
Limestone	0.20	0.20	0.20
Lysine	0.15	0.15	0.15
Methionine	0.20	0.20	0.20
Premix	0.25	0.25	0.25
Salt	0.30	0.30	0.30
TOTAL	100.0	100.0	100.0
Calculated analysis			
Crude protein	23.08	20.11	19.33
Ether extract	5.03	4.87	4.28
Crude fibre	3.06	3.95	3.42
Calcium	0.98	1.00	1.10
Phosphorus	0.47	0.40	0.51
Lysine	1.17	1.29	1.60
Meth + Cyst	0.87	0.82	0.51
ME (Kcal/kg)	2936	3000.8	3100.2

*每公斤膳食提供的预混料: 维生素A, 13000 I.U.; 维生素E, 5mg; 维生素D3, 3000I.U.; 维生素K, 3mg; 维生素B2, 5.5mg; 烟酸, 25mg; 维生素B12, 16mg; 氯化胆碱, 120mg; 锰, 5.2mg; 锌, 25mg; 铜, 2.6克; 叶

酸, 2mg; 铁, 5g; 泛酸, 10mg; 生物素, 30.5克; 抗氧化剂, 56mg。

表2 合欢树皮氨基酸组成

Amino acids	Composition (%)	*Reference level
Lysine	0.86	5.50
Arginine	1.65	1.00
Aspartic acid	2.00	-
Threonine	1.12	0.65
Histidine	3.45	0.30
Serine	0.78	-
Glycine	1.00	1.20
Alanine	3.00	-
Cysteine	4.11	0.35
Valine	0.65	0.82
Leucine	1.00	1.20
Phenylalanine	0.34	0.50
Tyrosine	0.10	0.45
Isoleucine	2.00	0.60
Proline	0.03	0.20
Methionine	0.02	0.35

*NRC (1994)

表3 饲喂不同水平ATSM的肉鸡血液学参数

Parameters	T1	T2	T3	T4	T5	SEM
PCV (%)	26.50 ^a	29.31 ^b	31.02 ^b	33.56 ^a	34.00 ^a	0.37
Hb (g/dl)	9.12 ^c	10.21 ^b	11.93 ^b	12.11 ^b	12.50 ^a	0.64
RBC × 10 ⁹ /μl	1.88 ^c	2.00 ^b	2.10 ^b	2.60 ^a	2.97 ^a	0.07
MCV (fl)	111.3 ^b	119.8 ^a	120.5 ^a	123.6 ^a	130.4 ^a	8.10
MCH (pg)	34.51 ^c	50.43 ^b	56.11 ^b	57.67 ^a	59.00 ^a	2.51
MCHC (g/dl)	29.80 ^b	35.60 ^a	39.00 ^a	39.10 ^a	40.03 ^a	0.88
WBC × 10 ³ /μl	20.41 ^b	20.62 ^b	22.74 ^b	22.88 ^b	30.04 ^a	0.12
Differentials (10 ³ /μl)						
Lymphocytes	10.45 ^c	14.08 ^b	15.44 ^b	18.71 ^b	20.04 ^a	1.96
Monocytes	0.07 ^c	1.11 ^b	1.20 ^b	1.26 ^b	1.72 ^a	0.01
Heterophils	4.23 ^b	5.06 ^a	5.40 ^a	5.89 ^a	6.11 ^a	0.41
Eosinophils	0.88 ^b	1.02 ^a	1.09 ^a	1.21 ^a	1.27 ^a	0.02

Means in the same row with different superscript are significantly different ($P<0.05$)

表4 喂食不同水平ATSM的肉鸡血清分析

Parameters	T1	T2	T3	T4	T5	SEM
Total protein (g/dl)	2.57 ^b	3.22 ^a	3.69 ^a	3.88 ^a	3.97 ^a	0.67
Albumin (g/dl)	1.34 ^b	1.55 ^a	1.91 ^b	2.00 ^a	2.03 ^a	0.02
Globulin (g/dl)	1.23 ^c	1.67 ^b	1.78 ^b	1.88 ^a	1.94 ^a	0.15
Creatinine (mg/dl)	0.15 ^c	0.45 ^b	0.81 ^a	0.87 ^a	0.91 ^a	0.01
Glucose (mg/dl)	196.1 ^c	204.3 ^b	234.1 ^a	241.5 ^a	250.6 ^a	4.33
Cholesterol (mg/dl)	101.4 ^a	98.6 ^b	90.4 ^b	89.4 ^b	87.5 ^b	2.87
Uric acid (mg/dl)	7.33 ^a	4.89 ^b	4.22 ^b	4.00 ^b	3.88 ^b	0.05
ALT (u/l)	74.1 ^a	70.5 ^b	61.6 ^b	58.1 ^b	50.7 ^b	1.45
AST (u/l)	300.7a	288.5 ^b	230.4 ^b	218.0 ^b	200.9 ^b	9.45

Means in the same row with different superscripts differ significantly ($P<0.05$)

表5 饲喂不同水平ATSM的肉鸡的抗氧化状态

Parameters	T1	T2	T3	T4	T5	SEM
MLA (U/mg Hb)	1.85 ^c	2.77 ^b	2.93 ^b	3.04 ^a	3.11 ^a	0.03
SDA (U/mg Hb)	35.7 ^b	39.8 ^a	40.7 ^a	43.5 ^a	45.3 ^a	1.21
GPx (U/mg Hb)	27.1 ^b	29.4 ^b	33.8 ^a	34.7 ^a	38.3 ^a	1.96
CAT (U/mg Hb)	54.2 ^a	45.7 ^b	42.5 ^b	41.6 ^b	40.1 ^b	0.52

Means in the same row with different superscripts differ significantly ($P<0.05$)

3 结果和讨论

实验日粮的近似组成(表1)表明, 它含有23.08%、20.11%和19.33%的粗蛋白; 能量为2936.0 kcal、3000.8 kcal和3100.2 kcal, 适用于初学者、种植者和精加工者。乙醚提取物的含量范围是(4.28–5.03%), 粗纤维的含量范围是(3.06–3.95%)。根据NRC(1994), 最接近的成分满足鸟类的营养需求。粗纤维和乙醚提取物范围也符合Teodora等人(2020)关于喂肉鸡Hermetia illucens粉的报告。实验饮食中的钙(0.98–1.10%)和

磷 (0.47 – 0.51%) 范围与 Fascina 等人 (2007) 的报告一致；阿杜库 (2004)。适当的喂养是牲畜生产管理的关键要素之一，为了使动物达到最佳状态，需要为它们提供适当的均衡饮食，其中包含所有必要的营养素 (Alagbe 和 Oluwafemi, 2019)。

合欢树皮的氨基酸组成如表2所示。结果显示存在苏氨酸 (1.12%)、亮氨酸 (1.00%)、赖氨酸 (0.86%)、缬氨酸 (0.65%)、甘氨酸 (1.0%)、苯丙氨酸 (0.34%)、组氨酸 (3.45%)、蛋氨酸 (0.02%)、丙氨酸 (3.00%)、丝氨酸 (0.78%)、脯氨酸 (0.03%)、天冬氨酸 (2.00%)、精氨酸 (1.65%)、酪氨酸 (0.10%)、异亮氨酸 (2.00%)、天冬氨酸 (2.00%，半胱氨酸 (4.11%)。样品中组氨酸浓度较高，酪氨酸浓度最低。氨基酸是基因表达和细胞信号转导调节所必需的蛋白质的组成部分 (Chzmruspollert 等人, 2004)。苯丙氨酸在胰岛素分泌和脂肪氧化中起着至关重要的作用 (Ma 等人, 2010)。赖氨酸可确保有效生产激素、酶和能量 (Bazer 等人, 2009)。丙氨酸和谷氨酸能够为身体提供健康的骨骼系统和能量生产, Marc 和 Wu (2009); 木村 (2010)。异亮氨酸有助于调节血糖 (Tan 等人, 2010; Yin 等人, 2010)。充足的精氨酸可确保健康的免疫系统，并维持体内的内脏器官 (Brosnan 和 Brosnan, 2010; Wu 等人, 2010)。丝氨酸和半胱氨酸分别作为神经调节剂和抗氧化剂发挥关键作用 (Wu et al., 2010; Wu et al., 2010; Baker, 2009)。蛋氨酸维持了鸟类肝脏、羽毛形成和卵子大小或产量的完整性 (McKnight 等人, 2010; Pali 等人, 2009)。喂食不同水平 ATSM 的肉鸡血液学参数如表3所示。PCV 值介于 (26.50 – 34.00%)、Hb (9.12 – 12.50 g/dl)、RBC 1.88 – 2.97 ($10^6/\mu\text{l}$)、MCV (111.2 – 130.4 fl)、MCH (34.01 – 59.00 pg) 和 MCHC (29.80 – 40.03 g/dl)。T3、T4 和 T5 组的 RBC、PCV、Hb、MCV、MCH 和 MCHC 值高于 T2 和 T1 组 ($P<0.05$)。与其他治疗组相比, T4 和 T5 组的 WBC 最高, 为 $20.41 – 30.04 (10^3/\mu\text{l})$ 。与其他治疗组相比, T1 组单核细胞 (0.07 – 1.72%)、淋巴细胞 $10.45 – 20.04 (10^3/\mu\text{l})$ 、嗜异性细胞 $1.23 – 6.11 (10^3/\mu\text{l})$ 和嗜酸性粒细胞 $0.88 – 1.27 (10^3/\mu\text{l})$ 最低 ($P<0.05$)。测量的血液学参数遵循相似的模式, 从 T1 显著增加到 T5 ($P<0.05$)。但是, 所有值都在正常鸟类的生理范围内 (Talebi 等人, 2005; Ibrahim, 2012; Subhadarsini 和 Silpa, 2020)。Islam 等人 (2004); Abdi Hachesoo 等人 (2011) 报告了 RBC 范围 ($2.9 – 3.5 \times 10^6/\mu\text{l}$), 这种变化可能只是年龄、性别、品种、环境、激素和营养差异的结果 (Fudge, 2000)。血液学指标用于疾病诊断以及血液损伤程度

(Nse Abasi 等人, 2014; Omokore 和 Alagbe 2019)。PCV 和 MCH 是诊断贫血的有用指标 Nse Abasi 等人 (2014); Alagbe (2019)。较高的红细胞水平表明血液中有足够的氧气, 这为体内有效的营养物质运输提供了空间 (Isaac 等人, 2013; Ugwuene, 2011)。白细胞通过产生抗体在免疫系统中发挥重要作用, 白细胞低的动物感染风险高 (Iwuji 和 Herbert, 2012; Isaac 等人, 2013)。

实验鸟类的血清生化指标如表4所示。与其他治疗组相比, T1 组的总蛋白范围 (2.57 – 3.97 g/dl)、球蛋白 (1.23 – 1.97 g/dls)、白蛋白 (1.34 – 2.03 g/dl), T1 和 T2 组的 ALT (50.7 – 74.1 u/l) 和 AST (200.9 – 300.7 u/l, $P<0.05$) 高于其余治疗组。与其他处理相比, T4 和 T5 的总蛋白值显著升高 ($P<0.05$), 这可能是由于 ATSM 中存在一些相关营养素。(Alagbe 等人, 2020)。血液中的白蛋白含量通常受到蛋白质短缺的影响, 然而, 报告的数值在 Subhadarsini 和 Silpa 报告的范围内 (2020)。Ibrahim (2012); Obajuluwa 等人 (2020); Olafadehan 等人 (2020); Livingston 等人 (2020) 报告, 球蛋白和尿酸的范围分别为 (1.6 – 1.9 g/dl) 和 (3.7 – 5.2 mg/dl)。这一结果也与 Obikaonu 等人 (2011) 和 Simaraks 等人 (2004) 的发现相一致。胆固醇、肌酐、尿酸、ALT 和 AST 值遵循相似的模式, 从 T1 到 T5 显著下降 ($P<0.05$)。然而, 所有值都在 Olafadehan 等人 (2020) 报告的范围内。较低的肌酐和尿酸水平是一个迹象, 表明喂食 ATSM 不会损害鸟类的肾脏。根据 Alagbe (2020), ATSM 含有多种矿物质、维生素和生物活性化学物质或次生代谢物 (单宁、皂苷、黄酮、生物碱、苯酚等), 这些物质对肉鸡的致死剂量范围内。据报道, 尿素水平还受膳食蛋白质质量、数量、出血时间的影响, 是诊断肾损伤的敏感生物标记物 (Akand 和 Odunsi, 2012)。ATSM 还可以作为一种降血脂物质, 因为它能够降低血液胆固醇, 从而预防心脏病 (Alagbe, 2020)。ALT 和 AST 是由于饲料中存在有毒物质而触发的血清酶 (Olabanji 等人, 2007; Oluwafemi 等人, 2020)。获得的结果表明, ATSM 不含可能阻碍鸟类一般表现的抗营养物质或有毒物质, 这一结果证实了 Abdel 等人 (2014) 的早期报告; Cho 等人 (2014); Alagbe (2017) 对肉鸡中植物性饲料添加剂的影响。

受 ATSM 影响的氧化状态如表5所示。与其他处理相比, T1 组的超氧化物歧化酶 [SDA; 35.7 – 45.3 U/mg Hb]、谷胱甘肽过氧化物酶 [GPx; 27.1 – 28.3 U/mg Hb]、过氧化氢酶 [CAT; 40.1 – 54.2 U/mg H-b] 和丙二醛 [MLA; 1.85 – 3.11 U/mg-Hb] 值最低 ($P<0.05$)。根据 Alagbe 等

人 (2019), ATSM含有能够清除自由基的抗氧化剂, 从而对动物提供全面保护。苯酚和类黄酮的存在可防止生物分子、超氧阴离子和脂质过氧自由基的氧化损伤 (Hollman, 2001; Ojeuuyi 等人, 2014)。Lin 等人 (2003) 也报告了同样的结果, 他们观察到植物性饲料添加剂的摄入导致血清抗氧化酶活性增加, MDA 浓度降低。相反, Lan 等人 (2013) 报告说, 血液中谷胱甘肽的浓度不受植物源性饲料添加剂的影响。

4 结论

据报道, 饲料添加剂 (植物提取物) 具有多种生物活性, 包括抗菌、抗真菌、抗病毒、抗蠕虫、抗氧化和免疫调节剂, 因为它们含有酚类、黄酮类、生物碱、单宁、皂苷、萜类等植物化学物质。它们相对便宜、安全、有效, 对持续使用没有任何副作用。在30毫升/升的水中使用ATSM已证明能够对身体及其新陈代谢提供全面保护, 防止因抗氧化剂的存在而产生的自由基, 并且对肉鸡的血液状况没有有害影响。

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