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## Effect of L-tryptophan on growth performance, blood parameters and immune-response of Friesian calves

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**ABSTRACT:** Aim of this study was to investigate the effect of L-tryptophan (TRP) supplementation on the immunoglobulin concentration, blood parameters, and growth performance of Friesian calves during pre-weaning period (105 days). A total of 30 newborn Friesian calves weighing  $33.42 \pm 1.9$  kg at birth were divided into three similar groups, 10 calves in each group. From birth up to weaning, calves in the first group (G1) were served as a control, while milk of calves in the second (G2) and third (G3) groups was supplemented with TRP at level of 2 and 4 g/calf/day, respectively. Results showed that live body weight of calves in G2 and G3 significantly increased by 8 and 9% at 5 week, 10 and 11% at 10, and 14 and 16% at 15 weeks of age compared with those in G1, respectively. The average daily gain (ADG) was significantly higher in G2 and G3 than in G1. Overall mean of plasma immunoglobulin (Ig) concentrations was significantly higher in G3 and G2 than in G1 at all ages, while overall mean of plasma Ig concentrations significantly increased from birth up to weaning. Haematological parameters had significantly higher values of red (RBCs) and white (WBCs) blood cells counts, PCV value, haemoglobin (Hb) concentration and neutrophils percentage in G2 and G3 than in G1. Plasma concentration of total proteins, albumin and globulin were significantly higher in G2 than in G1. Concentration of total lipids, creatinine, glucose and urea-N were significantly lower in G2 than in G1. It could be concluded that TRP supplementation in milk improved growth performance and immune-response of Friesian calves during the pre-weaning period.

**Keywords:** Friesian calves, tryptophan, growth performance, immunity, blood parameters

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## INTRODUCTION

Tryptophan, an essential amino acid (EAA) and 5-hydroxytryptamine (serotonin) precursor, has been shown to assist piglets cope with weaning stress, to enhance performance, lowering blood levels of stress-related hormones, and to regulate lying behavior (LeFloch and Seve, 2007; Lee *et al.*, 2019a). In addition to reduce anxiety and regulate social behavior and hunger, serotonin also has physiologic effects on glucose and lipid metabolism in the peripheral system, causing the release of insulin in response to glucose and adipogenesis under fed circumstances (Yabut *et al.*, 2019). Feed intake and growth performance was negatively affected by TRP lack. TRP is not deficient due to ruminal fermentation (Ma *et al.*, 2011; Lee *et al.*, 2019a). In ruminants, TRP was found to increase the activity and secretion of pancreatic  $\alpha$ -amylase, to increase the digestibility of starch by promoting the secretion of the intestinal hormone cholecystokinin (Leja-Szpak *et al.*, 2004; Jaworek *et al.*, 2004; Lee *et al.*, 2019a). It is well known that TRP is a constituent of niacin, a precursor of serotonin and melatonin, and it acts as antioxidant and has stress relieving properties (Lee *et al.*, 2019a; Ma *et al.*, 2011). It is widely known that immunological responses were affected by TRP metabolism. Proliferative arrest was caused by low amounts of tryptophan detected by T lymphocytes (Munn *et al.*, 2005). Supplementing 4.5 g/d of TRP via milk replacer might suggest some benefits on TRP uptake or implications in oxidative defenses of calves at weaning (Yeste *et al.*, 2020). L-tryptophan is a crucial component in metabolic, physiological, and organ development and growth (LeFloch and Seve, 2007 and Lee *et al.* 2019a).

The goal of this study was to investigate the effects of TRP on average daily gain, feed conversion, immunoglobulin concentration, some blood parameters and growth performance of Friesian calves from birth to weaning.

## MATERIALS AND METHODS

The present study benefited from contributions from the Sakha Animal Production Research Station, Animal Production Research Institute, Agricultural Research Center. Animal Care and Ethics Committee of Kafrelsheikh University in Egypt granted permission for this study to be carried out (license number: KFS1345/10).

### Animals and treatments:

A total of 30 Newborn Friesian calves with aver-

age live body weight (LBW) of  $33.45 \pm 1.8$  kg were divided into three similar groups (10 calves in each group) according to their live body weight at calving. Calves in all groups suckled their dam colostrums and transitional milk for the 1<sup>st</sup> three days, and then they were fed on whole milk, starter and berseem hay according to the recommended requirements of Animal Production Research Institute (1997) as shown in Table (1).

Calves in the 1<sup>st</sup> group were without any supplementation and served as a control (G1), while milk of those in the 2<sup>nd</sup> (G2) and 3<sup>rd</sup> (G3) were supplemented with TRP (Livzon Group, Fuxing Pharmaceutical, Ningxia, China) at level of 2 and 4 g/calf/day, respectively. The treatment period lasted from three days of age up to weaning. Milk of calves in treatment groups was given in morning milk during the whole pre-weaning period. Representative samples of feed-stuffs were chemical analyzed for CP, CF, EE, NFE and ash on DM basis according to the official methods of the AOAC (2000). Chemical composition of feed-stuffs is presented in Table 2.

### Experimental procedures:

Calves were artificially fed the whole milk in plastic bucket twice daily at 7 a.m. and 6 p.m. during the experimental period. Starter was offered to calves at the beginning of 3<sup>rd</sup> week of age once daily at 8 a.m., while berseem hay was offered to calves at 11 a.m. (Table 1). Water was available all the day round.

Live body weight and feed intakes from whole milk, starter, berseem hay were individually determined every week, then average daily gain was calculated at birth, 5, 10, 15 weeks of age. Also, feed conversion was calculated as the amounts of dry matter intake (DMI), crude protein intake (CPI) and total digestible nutrients intake (TDNI) required per 1 kg live weight gain.

### Blood sampling:

Blood samples were collected from all calves biweekly for immunoglobulin determination and for haematological and plasma biochemicals at the weaning. Blood samples (5 ml) were collected before morning feeding and drinking from jugular vein using heparinized vacutainer tubes. Blood samples were centrifuged at 4000 rpm for 15 minutes and plasma was carefully separated and stored at -20 °C until analysis.

**Table 1.** Amounts daily of feedstuffs (kg/head) fed to calves during suckling period.

Feedstuff (kg/h)	Age (week)														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Whole milk	4.0	4.5	5.0	5.5	6.0	6.0	5.5	4.5	4.0	3.5	3.0	2.5	2.0	1.5	1.0
Starter*	-		0.25		0.50		0.75		1.00		1.25		1.50		1.75
Berseem hay	-		0.25		0.75		0.75		1.0		1.25		1.50		1.75

**Table 2:** Chemical analysis of feed stuffs (% on dry matter basis).

Item	Starter	Whole milk	Berseem hay
Dry matter, DM	91.28	12.56	88.78
Organic matter, OM	90.54	93.68	88.12
Crude protein, CP	17.24	24.26	14.45
Crude fiber, CF	5.96	0.00	24.74
Ether extract, EE	4.91	30.64	6.26
Nitrogen free extract	62.38	39.25	42.97
Ash	9.46	6.32	11.88

Haematological parameters including count of red (RBCs), white blood cells (WBCs), hemoglobin (Hb) concentration and packed cell volume (PCV%) were determined in fresh whole blood using fully digital haematology counter (Abaxis, Union City, CA., USA).

Bovine IgG ELISA kits were used to determine the concentration of immunoglobulin (IgG) in blood plasma, following the manufacturer's instructions (Alpha Diagnostic International, Texas, USA and Kamiya Biomedical Company, Seattle, Washington, USA, respectively).

At weaning, concentration of total proteins, albumin, urea-N, creatinine and glucose in blood plasma were determined using commercially available kits (Diagnostic System Laboratories, Inc., USA).

#### Statistical analysis:

Statistical analysis of the obtained data was performed to study the effect treatment on parameters studied at each age, except parameters of immunoglobulin were tested by factorial design (treatment x time). The statistical analysis was carried out applying the package of SAS (2004) according to the following models:  $Y_{ij} = \mu + H_i + e_{ij}$ , Where:  $Y_{ij}$  = the studied dependent variable,  $\mu$  = the overall mean,  $H_i$  = the effect of treatment (i, 1 & 2) and  $e_{ij}$  = random residual effect.

However, the effect of treatment, time, and their interaction was tested according to the following model:  $Y_{ijk} = \mu + H_i + B_j + HB_{ij} + e_i$ , Where:  $Y_{ijk}$  = the

studied dependent variable,  $\mu$  = the overall mean,  $H_i$  = the effect of treatment (i, 1 & 2),  $B_j$  = the effect of time  $HB_{ij}$  = Interaction due treatments x time and  $e_{ij}$  = random residual effect.

Duncan Multiple Ranges Test was used to see if there were any significant variations between the means at  $P < 0.5$  (Duncan, 1955).

## RESULTS AND DISCUSSION

### Growth performance:

#### Live body weight (LBW) and average daily gain (ADG):

Data in Table 3 showed that effects of TRP on LBW and ADG were significant. Live body weight of calves in G2 and G3 was significantly higher by 8 and 9% ( $P < 0.05$ ) at 5 weeks, 10 and 11% ( $P < 0.01$ ) at 10 weeks, and 14 and 16% at 15 weeks (weaning) compared with those in G1, respectively. Also, ADG during different age intervals was significantly higher in G2 and G3 than in G1 (Table 3). The ADG increased by 19 and 30%, 17 and 14%, 27 and 31%, and 21 and 25% in G2 and G3 compared with those in G1 at 0-5, 6-10, 11-15, and 0-15 weeks, respectively.

In accordance with our results 5-hydroxy-l-tryptophan improved LBW of calves as compared to control (Hernández-Castellano *et al.*, 2018). Feeding dietary RPT has been shown to not only increase growth performance of lambs (Van *et al.*, 2008) but also improve N-utilization in goats (Lee *et al.*, 2019b) and average daily gain (ADG) and N-utilization in cashmere goats (Ma *et al.*, 2011).

**Table 3:** Live body weight and average daily gain of calves in the experimental groups.

Age (week)	G1 (Control)	Tryptophan	
		(G2, 2g/h/d)	(G3, 4g/h/d )
<b>Live body weight (kg):</b>			
At birth	33.65±1.5	33.85±1.3	32.85±2.1
5	53.55±1.4 <sup>b</sup>	57.60±1.6 <sup>ab</sup>	58.60±1.5 <sup>a</sup>
10	74.10±3.2 <sup>b</sup>	81.62±3.6 <sup>a</sup>	81.90±3.5 <sup>a</sup>
15 (at weaning)	97.50±4.5 <sup>b</sup>	111.40±4.6 <sup>a</sup>	112.75±4.7 <sup>a</sup>
<b>Average daily gain (Kg):</b>			
Birth ~ 5	0.57±0.058 <sup>b</sup>	0.68±0.052 <sup>a</sup>	0.74±0.052 <sup>a</sup>
6 ~ 10	0.59±0.039 <sup>b</sup>	0.69±0.045 <sup>a</sup>	0.67±0.045 <sup>a</sup>
11 ~ 15	0.67±0.034 <sup>b</sup>	0.85±0.025 <sup>a</sup>	0.88±0.025 <sup>a</sup>
Birth ~ 15 (weaning)	0.61±0.027 <sup>b</sup>	0.74±0.034 <sup>a</sup>	0.76±0.034 <sup>a</sup>

<sup>a</sup> and <sup>b</sup> Within the same row, group means marked by the same superscripts are not substantially different at (P<0.01).

**Table 4:** Daily feed intake and feed conversion of calves during birth to weaning.

Item	Control (G1)	Tryptophan (G2)	Tryptophan (G3)
<b>Average daily feed intake (kg/head/day)</b>			
Whole milk	2.12	2.12	2.12
Starter	0.91	0.94	0.95
Fresh berseem	0.86	0.89	0.91
TDMI (kg)	1.94±0.06	2.0±0.08	2.02±0.08
TDNI	1.45±0.008	1.50±0.01	1.52±0.009
DCPI	0.343±0.007	0.345±0.005	0.347±0.005
<b>Feed conversion (kg/ kg LBW)</b>			
DM	3.18±0.07 <sup>a</sup>	2.70±0.08 <sup>b</sup>	2.66±0.08 <sup>b</sup>
TDN	2.38±0.06 <sup>a</sup>	2.03±0.08 <sup>b</sup>	2.00±0.07 <sup>b</sup>
DCP	0.56±0.01 <sup>a</sup>	0.47±0.01 <sup>b</sup>	0.46±0.01 <sup>b</sup>

<sup>a</sup> and <sup>b</sup> At (P<0.01), group means designated by the same superscripts are not significantly different within the same row.

### Intake of feed and feed conversion:

Average daily feed intakes by Friesian calves are shown in Table 4. Total DM intake was almost similar for the two groups, while TDN and DCP intakes were insignificantly higher in G2 and G3 than in G1. The increase of TDN and DCP intakes was in parallel with increasing levels of TRP. These results agreed with those reported by Lee *et al.*, (2019a), who found that feed intake and development performance may be impaired by a lack of TRP.

Results in Table 4 revealed that TRP supplementation significantly improved feed conversion of Friesian calves as amount (kg) of DM, TDN and DCP required per kg gain compared with control group.

Feed conversion as DM, TDN and DCP improved (P<0.05) by 17.78, 17.24 and 19.15% in G2, and 19.55, 19.00 and 21.74% in G3 compared with G1, respectively. These results are attributed to increasing ADG as well as decreasing feed intakes in G2 and G3 in comparing with G1 (Table 4). Similar results were

reported by Jo *et al.*, (2021).

### Concentration of total immunoglobulins (Ig, mg/ml) in blood plasma:

Results shown in Table 5 revealed that overall mean of plasma immunoglobulins concentrations at different ages of pre-weaning period was significantly higher in G2 and G3 than in G1. Total immunoglobulins concentrations in plasma of calves in G2 and G3 increased at a range from 10 to 19% as compared to G1. Age had a significant effect on the concentrations of total immunoglobulin in the plasma of calves during pre-weaning period. Overall mean of plasma immunoglobulin concentrations increased gradually with advancing age from birth up to weaning in all groups. In this respect, Jezek *et al.* (2009) reported that concentration of IgG increased from 12 to 20 weeks of age. The immune system of newborn calves is not able to respond at the level of adult animals and they are more susceptible to infection in this period. So, calves must be ingested colostrum after birth, as



**Table 5:** Concentration of total immunoglobulins (Ig, mg/ml) in blood plasma of calves in the experimental groups as affected by treatment, age, and their interaction.

Age (week)	Control (G1)	Tryptophan treatment		Overall means
		(G2, 2 g/h/d)	(G3, 4 g/h/d)	
1	24.42±0.99	27.95±1.11	27.95±1.11	26.77±0.98 <sup>F</sup>
3	26.25±1.15	30.54±1.12	30.54±1.12	29.11±1.11 <sup>D</sup>
5	29.45±0.98	32.56±0.92	32.56±0.92	31.22±0.87 <sup>D</sup>
7	32.23±1.14	36.12±0.96	36.12±0.96	34.82±0.98 <sup>E</sup>
9	33.45±1.19	37.58±1.20	37.58±1.20	36.20±1.12 <sup>CE</sup>
11	35.57±1.21	39.57±1.13	39.57±1.13	38.24±1.14 <sup>BC</sup>
13	37.89±1.31	42.65±1.27	42.65±1.27	41.06±1.21 <sup>AB</sup>
15	39.35±0.96	44.82±0.87	45.95±1.11	43.37±0.97 <sup>A</sup>
<b>Overall means</b>	32.33±1.14 <sup>b</sup>	36.47±1.05 <sup>a</sup>	36.62±1.20 <sup>a</sup>	

<sup>a</sup> and <sup>b</sup> At (P<0.01), group means designated by the same superscripts are not significantly different within the same row.

A, B,... and F At (P<0.01), group means designated by the same superscripts are not significantly different within the same column.

soon as possible, to effectively absorb colostral immunoglobulin G (Rajala and Castren, 1995).

The present results of plasma IgG (24-28 mg/mL) at the 1<sup>st</sup> wk of age indicated the accepted serum limit of the IgG (at least 10 g/L within 24-48 h postpartum) as reported by Besser *et al.* (1991).

Tryptophan 2,3- dioxygenase is a liver enzyme that is activated by TRP and metabolic steroids and is highly specific for the substrate TRP (Sainio 1997). The conflicting regulation mechanisms for the two enzymes, a food sub strate in one case and an immune-activating substance in the other, show that tryptophan metabolism may be controlled differently in health and sickness by a physiological switching mechanism. However, the physiological significance of this shift in TRP catabolism is still debated (Moffett and Namboodiri, 2003).

The TRP (5-hydroxytryptamine) is produced by enterochromaffin cells in the stomach, but it is also produced by platelets (Lesurtel *et al.*, 2006) and mammary epithelial cells (Hernandez *et al.*, 2009). Peripheral TRP is involved in a variety of physiological processes, such as calcium homeostasis, the control of glucose (Watanabe *et al.*, 2014; Laporta *et al.*, 2015), and lipid metabolism (Weaver *et al.*, 2016; Hernández-Castellano *et al.*, 2017).

In addition, TRP regulates several innate immune pathways, including mastocyte adhesion and chemotaxis in humans and mice (Kushnir-Sukhov *et al.*, 2006), eosinophil chemotaxis in humans (Boehme *et al.*, 2004), and the stimulation of cytolytic activity (Hernandez *et al.*, 2010) and proliferation of natural killer cells in humans (Boehme *et al.*, 2004). Sero-

tonin also impacts the adaptive immune system by boosting T- and B-cell proliferation (Serafeim *et al.*, 2002; León-Ponte *et al.*, 2007). We suggested that supplementing colostrum and milk with TRP might help the calf's immune system grow and impact several metabolic pathways during the early weeks of life, based on the varied effects of TRP on the immune system. To enhance immunity, calves must be fed IgG-containing colostrum within 12 hours of birth (Lopez and Heinrichs, 2020). This will benefit calves' growth, health, eventually, and productivity. Marcela *et al.* (2021) show that reducing heat stress in dairy calves early in life can increase peripheral 5-hydroxytryptamine metabolism and assist the development of humoral immune responses, both of which can help with disease resistance.

#### Haematological parameters:

Results of haematological parameters presented in Table 6 revealed significantly higher values of RBCs and WBCs counts, PCV value (P<0.01), haemoglobin (Hb) concentration and neutrophils percentage (P<0.05) in G2 and G3 than in G1. However, percentages of monocytes, basophils, eosinophils and lymphocytes were not affected by TRP supplementation. Counts of RBCs and WBCs and percentages of neutrophils increased in G2 and G3 compared with G1 by 13.31, 14.89, and 4.24% in G2 and, 15.71, 16.52, and 9.23% in G3, respectively. The corresponding increase in PCV value and Hb concentration were 15.57 and 17.32% in G2 and 18.87 and 19.18% in G3, respectively. The observed improvements of haematological traits observed in calves fed tryptophan may be due to improvement in immune system responsiveness. Calves' innate and adaptive immune systems

**Table 6:** Haematological parameters in blood of calves in the experimental groups at weaning age.

Items	Control (G1)	Tryptophan treatment	
		(G2, 2 g/h/d)	(G3, 4 g/h/d)
Red blood cells ( $\times 10^6/\text{mm}^3$ )	8.34 $\pm$ 0.25 <sup>b</sup>	9.45 $\pm$ 0.32 <sup>a</sup>	9.65 $\pm$ 0.33 <sup>a</sup>
Package cell volume (%)	29.55 $\pm$ 1.42 <sup>b</sup>	34.15 $\pm$ 1.27 <sup>a</sup>	35.10 $\pm$ 1.35 <sup>a</sup>
Haemoglobin (g/dL)	10.22 $\pm$ 0.52 <sup>b</sup>	11.99 $\pm$ 0.56 <sup>a</sup>	12.18 $\pm$ 0.65 <sup>a</sup>
WBCs ( $\times 10^3/\text{mm}^3$ )	10.432 $\pm$ 0.32 <sup>b</sup>	11.985 $\pm$ 0.40 <sup>a</sup>	12.155 $\pm$ 0.43 <sup>a</sup>
Neutrophils (%)	40.51 $\pm$ 0.9 <sup>b</sup>	43.85 $\pm$ 0.9 <sup>a</sup>	44.25 $\pm$ 1.1 <sup>a</sup>
Basophils (%)	0.31 $\pm$ 0.07	0.29 $\pm$ 0.06	0.28 $\pm$ 0.05
Eosinophils (%)	2.35 $\pm$ 0.22	2.28 $\pm$ 0.25	2.38 $\pm$ 0.25
Lymphocytes (%)	53.87 $\pm$ 2.9	50.60 $\pm$ 2.5	50.45 $\pm$ 2.5
Monocytes (%)	3.21 $\pm$ 0.36	3.65 $\pm$ 0.38	3.67 $\pm$ 0.42

<sup>a</sup> and <sup>b</sup> At (P<0.01), group means designated by the same superscripts are not significantly different within the same row.

**Table 7:** Biochemical parameters in blood plasma of calves at weaning age.

Items	Control (G1)	Tryptophan treatment	
		(G2, 2 g/h/d)	(G3, 4 g/h/d)
Total protein (g/dl)	7.75 $\pm$ 0.35 <sup>b</sup>	8.86 $\pm$ 0.33 <sup>a</sup>	8.96 $\pm$ 0.33 <sup>a</sup>
Albumin (g/dl)	3.73 $\pm$ 0.31 <sup>b</sup>	4.21 $\pm$ 0.24 <sup>a</sup>	4.04 $\pm$ 0.25 <sup>a</sup>
Globulin (g/dl)	4.02 $\pm$ 0.23 <sup>b</sup>	4.65 $\pm$ 0.21 <sup>ab</sup>	4.92 $\pm$ 0.20 <sup>a</sup>
Total lipids (mg/dl)	211.3 $\pm$ 11.2 <sup>a</sup>	176.2 $\pm$ 10.1 <sup>b</sup>	171.8 $\pm$ 11.5 <sup>b</sup>
Glucose (mg/dl)	71.64 $\pm$ 3.17 <sup>a</sup>	61.68 $\pm$ 3.50 <sup>b</sup>	60.45 $\pm$ 3.35 <sup>b</sup>
Creatinine (mg/dl)	1.81 $\pm$ 0.08 <sup>a</sup>	1.51 $\pm$ 0.06 <sup>b</sup>	1.49 $\pm$ 0.09 <sup>b</sup>
Urea-N (mg/dl)	60.85 $\pm$ 2.5 <sup>a</sup>	51.87 $\pm$ 2.2 <sup>b</sup>	50.45 $\pm$ 2.4 <sup>b</sup>
AST (U/L)	53.57 $\pm$ 5.64	52.54 $\pm$ 4.78	50.75 $\pm$ 5.67
ALT (U/L)	25.48 $\pm$ 4.65	22.55 $\pm$ 3.35	22.15 $\pm$ 3.25

<sup>a</sup> and <sup>b</sup> At (P<0.01), group means designated by the same superscripts are not significantly different within the same row.

may be affected by TRP supplementation, perhaps by macrophage activation and the subsequent release of immunological active chemicals (Hernández-Castellano *et al.*, 2018).

### Biochemical parameters:

Data presented in Table 7 showed that both TRP levels significantly (P<0.05) increased total proteins and albumin concentrations in blood plasma of calves by 14.32 and 12.87% in G2 and by 15.61 and 8.31% in G3 compared with control, respectively. Globulin concentration was significantly (P<0.05) increased only by higher TRP level (G3) than the control group. However, concentration of total lipids, glucose, creatinine and urea-N were significantly lower (P<0.01) in G2 and G3 than in G1. On the other hand, plasma AST and ALT activities were almost similar in both groups. The present values of plasma total protein are within the normal range and in agreement with those obtained by several investigators (Al-Kudsi *et al.*, 2008; Lee *et al.*, 2008) on calves. In agreement with our results Yeste *et al.* (2020) and Yabut *et al.* (2019) found that the TRP group had a propensity to have lower plasma glucose concentrations after feeding.

The decreased glucose concentration in TRP calves might be explained by the fact that serotonin, a TRP derivative, has an insulin-releasing action under fed circumstances, and higher insulin levels in the TRP group would result in quicker glucose absorption in the peripheral tissues (Yabut *et al.*, 2019).

### CONCLUSION

These findings suggest that TRP supplementation throughout the nursing period increased the growth performance and immunological response of Friesian calves without having a negative impact on haematological and biochemical parameters during pre-weaning period.

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### CONFLICT OF INTEREST

None declared.

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