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NONRUMINANT NUTRITION

Physiological, immunological and microbial effects of soybean bioactive peptides and vitamin E supplementing to broiler diet

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ABSTRACT. The physiological, immunological, and microbiological traits of 180 Ross-308 broiler chickens were examined in relation to supplementary soybean bioactive peptide (SBP) levels and vitamin E. Group 1 was the control group, Groups 2, 3, and 4 were given a basal diet along with 2.5, 5, and 7.5 g SBP kg^{-1} of diet, respectively, and Group 5 was given a basal diet together with 250 mg of vitamin E kg^{-1} of diet. An improvement in the globulin, albumin-to-globulin ratio, total antioxidant activity (except 7.5 g), SOD, and GSH-PX (except 7.5 g) activities in SBP treatment groups compared to the control. In comparison with control, feeding SBP improved bursa index, IgA, and IgG concentrations in blood serum. Feed supplementation with 5 g SBP significantly increased relative weight of spleen and IgM concentrations. Feeding SBP increased lactic acid bacteria in the ileum and caecum while decreasing *E. coli* counts in the ileum as compared with control. None of the selected serum biochemical indices, immune-related parameters, and total bacterial count were statistically different between the vitamin E group and the control. Therefore, 5 and 7.5 g of SBP kg^{-1} in a broiler diet was able to boost antioxidant status, antibacterial activity, and birds' immune response.

Keywords: antioxidant status; Ross-308; SBP; immunity; serum; bacteria.

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Introduction

Utilizing soybean as a feed source for broilers can enhance production efficiency due to its high nutritional content and balanced amino acid composition (Rutkowski, Kaczmarek, Hejdysz, Nowaczewski, & Jamroz, 2015). Antinutritional components in soybean meal include phytate, lectin, and protease inhibitors(Wang et al., 2020a). These elements will harm an animal's digestive system's structure, decrease the utilization of proteins and minerals, prevent animals from being fed, and upset the balance of intestinal flora (Zhou, Ringø, Olsen, & Song, 2018). As a result, a practical technique to lessen the antinutritional elements is required to ensure the intestinal proliferation of beneficial microbes.

An essential method for improving the use of cereal proteins is through the use of bioactive peptides (Li-Chan, 2015). Therefore, it is possible produce high-quality short peptides with physiological, regulatory, or nutritional purposes in poultry(Hou, Wu, Dai, Wang, & Wu, 2017). There are numerous mechanisms to create bioactive peptides, including enzymatic digestion (Zenezini et al., 2016). Alternatively, they could be actively created through microbial fermentation(Xu, Hong, Wu, & Yan, 2019), in vitro enzymatic, alkali, or acid hydrolysis (Rizzello et al., 2016) .

Vitamin E is frequently added to poultry feed as an antioxidant and immune booster(Liu et al., 2019; Yang et al., 2020). To compare the bioactivity of soybean peptides in this investigation, vitamin E was added. The National Research Council (NRC) states that broiler chicken needs 10 IU of vitamin E per kilogram of feed (National Research Council [NRC], 1994).

Numerous investigations have revealed that bioactive peptides have significant antibacterial potency (Roque-Borda et al., 2021; Jiaxu et al., 2022), antioxidant activity(Landy, Kheiri, & Faghani, 2021), and immunomodulatory properties (Osho, Xiao, & Adeola, 2019; Landy, Kheiri, & Faghani, 2021). In this context, Wang et al. (2020b) reported that the peptide-fed birds exhibited lower levels of Escherichia coli in their feces. According to Salavati, Rezaeipour, Abdullahpour, and Mousavi (2021) study, which looked at how bioactive peptides produced from sesame meal affected immunological response and certain serum metabolites, these peptides can be utilized as a supplement to broiler chicken diets without having a deleterious impact on the immune system. While Alahyaribeik, Nazarpour, Tabandeh, Honarbakhsh, and Sharifi (2022) reported that adding feather bioactive peptides to water at a rate of 50 mg L^{-1} resulted in lower serum levels of total cholesterol, triglycerides, and low-density lipoprotein in broilers. According to Xie et al. (2020), when two antibacterial peptide and antibiotic combinations were compared, their effects on immune function, antioxidant capacity, serum biochemical indicators, and microorganisms in broilers were examined. It was discovered that these indicators were favorable to the peptide treatments.

Currently, there is a lack of information on comparing the bioactivity of soybean peptides with vitamin E on Physiological, immunological, and microbial criteria on broilers. Therefore, this study aimed to evaluate the effects of soybean bioactive peptides and vitamin E supplementation on the determination of some physiological, immunological and microbial parameters in broiler. We hoped that this experiment would provide new knowledge about the biological bounce of soybean peptides and provide technical support for the application of SBP in broiler production.

Material and methods

Soybean bioactive peptide and vitamin E preparation and analysis

SBP was obtained from Vanavaran Novin Joestar Biotechnology, Tehran, Iran (in the form of yellow-brown powder). While vitamin E was obtained from American SOLGAR Company - Manhattan. The molecular weight distribution of SBP was determined using a Superdex peptide HR 10/300 GL column coupled with an HPLC system by a method (Karimzadeh & Teimouri Yansari, 2016).

Experimental birds and design

The experiment was conducted in the Poultry Field, College of Agriculture, University of Basrah from January 19 to February 25, 2022, for a total of 5 weeks (35 days). A total of 180 day-old broiler chicks (Ross-308) were randomly assigned to five treatment groups, each of which contained three replicates of 12 birds. The chicks in the control group were given no supplement (basal diet), whereas in supplement treatment groups chicks received 2.5, 5, and 7.5 g SBP kg-1 diet, and 250 mg kg-1 vitamin E was added to the basal diet. A broiler starter diet (1–21 days) and a broiler grower diet (22–35 days) were given to the chicks. All of the chicks were kept during the experiment in the same optimal humidity, temperature, and lighting levels as advised by the Ross-308 broiler manufacturer's recommendation. Basal diets were created in accordance with the recommendations to satisfy Ross-308 broiler's typical nutritional needs (Table 1). During the trial, birds had unrestricted access to food and water.

Table 1. Ingredients and composition of the basal diet (%).

Ingredients and composition	Starter (1 to 21 days)	Grower (22 to 35 days)	Calculated analysis***	Starter	Grower
Yellow corn	53.0	54.0	ME, kcal kg^{-1}	2988.49	3054.29
Wheat	6.00	9.00	Crude protein	23.03	21.55
Soybean meal 48%CP	32.0	29.0	Crude fiber	3.78	3.64
Broiler concentrate*	5.30	4.00	Calcium	1.24	1.10
Sunflower oil	1.00	1.50	Available phosphorus	0.46	0.40
Dicalcium phosphate	0.50	0.50	Lysine	1.34	1.11
Limestone	0.50	0.50	Methionine	0.50	0.42
DL-Methionine 99%	0.17	0.11	Methionine + Cystine	0.78	0.72
L-Lysine-HCl 98%	0.23	0.09	Energy: protein	129.76	141.73
Premix**	1.00	1.00			
Common salt	0.30	0.30			
Total	100	100			

*Super concentrate contains the following: 40% CP, 3.8% CF, 10% calcium, 4.5% available phosphorus, 1.8% lysine, 0.55% methionine, 2.89% methionine + cysteine,. Metabolizable energy 2800 kcal/kg, 0.14% Sodium. **premix supplied the following per kilogram of diet: Vitamin A, 6 mg; vitamin D3, 0.15 mg; vitamin E, 40 mg; vitamin K3, 4 mg; B1, 3 mg; B2, 12 mg; B6, 10mg; vitamin B12, 0.04 mg; niacin, 60mg; choline chloride, 700 mg; calcium D-pantothenate, 20 mg; folic acid, 2 mg; Biotin, 0.2 mg; Iron, 90 mg; Copper, 30 mg; Manganese, 120 mg; Zinc, 140 mg; Iodine, 4 mg; Selenium, 0.8 mg; Calcium, 30.8%. ***Calculated analysis : NRC (1994) feed ingredient tables were used for calculation.

Sample collection and preparation

Each replication included three chickens chosen at random. Birds' blood was drawn from the wing vein and placed in standard tubes without heparin. The serum was then separated by centrifugation at 3000 rpm for 15 min. at 4 °C, and it was then kept at 20 °C until it was used in biochemical assays. After The birds have been slaughtered, the ileum and caecum were dissected over a 10-cm length, and the contents were aseptically collected into a 2-mL safe lock tube. The ceca contents were immediately frozen at −30°C to use for detecting the counts of bacteria.

Determination of serum biochemical parameters

Biochemical indicators of blood serum (Total protein, albumin, globulin, glucose, total cholesterol and triglycerides) were estimated using commercially available kits supplied by BIOLABO-SAS Company, and the assay was done according to the manufacturer's instructions. The biochemical analysis was done using a spectrophotometer. The Enzyme-Linked Immune Sorbent Assay was used to estimate the activity of antioxidant enzymes in serum, including superoxide dismutase (SOD) and glutathione peroxidase (GSH-PX) enzymes using the standard ready-made kit from by IBM International GMBH, Germany. The total antioxidant activity was determined using Bioassay systems commercial kit (Re et al. 1999).

Detection of immunoglobulin in serum and immune organ index

To assess the antibodies response, the contents of IgG, IgM, and IgA in chicken serum were determined by ELISA kit (Henyuan Biotechnology Co., Ltd., Shanghai, China) in accordance with the manufacturer's recommendations and earlier studies (Hirakawa, et al., 2020; Al-Kahtani, Alaqil, & Abbas, 2022). On d 35, after the birds have been slaughtered, the spleen and bursa of Fabricius were collected and weighed to calculate the immune organ index (g kg^{-1}).

Gut microflora counts

Samples from the ileum and caecum were diluted into nine successive incisions using sterile tubes in order to count the number of bacteria. Dilutions were then applied to several agar mediums: Dilutions were plated on agar medium to count the total bacterial count, Lactic acid bacteria were cultivated on MRS agar, and E. coli were cultivated on MacConkey agar. Then the petri dishes were placed in the incubator at 37°C. Colonies were enumerated after incubation, and the amount of bacteria was represented [l g (CFU g^{-1})] as an algorithm related to the overall bacterial community in 1 g intestinal contents.

Statistical analysis

The data were statistically analyzed by one-way ANOVA procedure using the SPSS software (Statistical Package for Social Sciences, 2022) program. The significant differences among the treatment groups were analyzed by Duncan’s (Duncan, 1955) multiple-range tests at ($p \leq 0.05$) level of significance.

Results and discussion

Molecular weight distribution of soybean bioactive peptides

The results of the analysis of soybean bioactive peptides in the present study showed that the molecular weights of soybean bioactive peptides were in the range of 180-2500 Da for all parts, and the main fraction of peptides (68.85 %) was constituted by di-/tripeptides (180-500 Da) followed by oligopeptides/polypeptides (26.43%) with molecular weight in the range of 500 to >2500 Da, and amino acids (4.72%) (< 180 Da) (Table 2).

Table 2. Molecular weight distribution of soybean bioactive peptide.

Molecular weight range (Da)	Peptide fraction (%)
Di/Tri peptides (small peptides) (180 – 500)	68.85
Oligopeptides /Polypeptides (500 to >2500)	26.43
Amino acids (<180)	4.72

The biochemical metabolites

Blood biochemical indices are frequently used to monitor changes in organ function and metabolism in bird groups (Liu et al., 2015). Table 3 displays the impact of dietary supplementation with vitamin E and soybean bioactive peptide (SBP) on the broilers' serum metabolites. The dietary groups had no significantly effect ($p \geq 0.05$) on glucose, total serum protein, cholesterol, or triglycerides. But, a significant difference ($p \leq 0.05$) was seen in serum albumin, globulin, and albumin to globulin ratio. The highest level of globulin was obtained in the groups supplemented with 2.5, and 7.5 g of SBP kg^{-1} feed as compared with the control and Vit. E groups. Meanwhile, the albumin level was decreased in the groups supplemented with 2.5, and 7.5 g of SBP kg^{-1} feed. This results in an improvement in the albumin-to-globulin ratio in the peptide treatment groups compared to the control and Vit. E groups; as a result, the decrease in albumin-to-globulin ratio may signify an improvement in the birds' immunity.

Table 3. Some serum biochemical indices of broilers fed soybean bioactive peptide and vitamin E.

Parameters	Control	Soybean bioactive peptide g kg^{-1} diets			Vit. E	SEM	p <
		2.5	5.0	7.5	250 (mg kg^{-1})		
Glucose (g 100 mL^{-1})	220.83	195.58	230.66	204.55	256.60	12.59	0.643
Total Protein (g 100 mL^{-1})	3.82	3.78	3.92	3.96	3.59	0.05	0.223
Albumin (g 100 mL^{-1})	1.94^{a}	1.50^{b}	1.67^{ab}	1.47^{b}	1.65^{ab}	0.06	0.05
Globulin (g 100 mL^{-1})	1.86^{c}	2.25^{a}	2.23^{ab}	2.47^{a}	1.93^{bc}	0.07	0.008
Albumin to Globulin ratio	1.04^{a}	0.68^{bc}	0.75^{bc}	0.60^{c}	0.85^{ab}	0.04	0.05
Cholesterol (mg dL^{-1})	161.16	154.43	162.99	150.44	154.38	4.700	0.482
Triglycerides (mg dL^{-1})	89.91	82.98	85.27	83.80	91.05	1.554	0.385
Total antioxidant activity	155.33^{b}	187.00^{a}	191.33^{a}	180.0^{ab}	$6\ .178^{ab}$	4.54	0.05
SOD (U L^{-1})	4.96^{b}	6.76^{a}	6.84^{a}	6.55^{a}	6.30^{ab}	0.245	0.05
GSH-PX(U L^{-1})	7.70^{b}	9.70^{a}	9.46^{a}	8.50^{ab}	8.99^{ab}	0.262	0.05

SEM = Standard error of mean; $^{a-c}$ = Means within the same raw with different superscripts letters are significantly different ($p \leq 0.05$).

The variation in the concentration of albumin and globulin in the blood serum between the experimental treatments may be due to the variation in the concentrations of total protein (which differed mathematically without any significant difference), as the concentrations of total protein increased in the blood serum of birds added to their diets with soybean peptides compared to the two control treatments, As a result of the microbial balance and the improvement of intestinal conditions, which improved the ability to absorb nutrients as a result of the contribution of lactic acid bacteria to the secretion of digestive enzymes to break down proteins and convert them into amino acids, and thus their concentration increases in the form of total protein in the blood serum. Improving the level of biochemical indicators In birds fed diet containing soybean peptides could be due to the readiness of nutrients that these protein fractions, this may be related to the low molecular weight of these peptides (Table 2).

The highest Total antioxidant activity of serum obtained in the groups which received 2.5, 5 g of SBP kg^{-1} feed. According to Ren et al. (2012), the total antioxidant activity is made up of a variety of antioxidant enzymes and the biomolecules they are intricately related to. In this regard, Landy, Kheiri, and Faghani (2020) reported that the addition of bioactive peptides derived from cottonseed to the diet of broilers significantly increased the total antioxidant activity of serum. Additionally, broilers treated with an excessive amount of vitamin E gained the highest total antioxidant capacity (Landy, Kheiri, & Faghani, 2021). Soybean peptides may have the ability to confer hydrogen from the amino acids in them. As well, the antioxidant peptides with a high content of essential amino acids such as Trp, Tyr, and Phe have the potential to confer electrons (Girgih et al., 2015).

In the current study, SBP supplementation in broiler diets increased SOD and GSH-Px enzyme activities in blood serum. SOD and GSH-Px can scavenge reactive oxygen type and thus function as antioxidants. SOD initially breaks down superoxide into hydrogen peroxide, which is then broken down further into water by a number of enzymes, including GSH-Px (Wang et al., 2008). Our findings supported what Zhao et al. (2022) who reported on the improvement of antioxidant indicators (SOD, GSH-Px) for laying hens when short peptides were introduced at various amounts (0, 1.5, 3.0, 4.5, and 6.0) g kg^{-1} of the laying hens' food during the starter and grower periods.

Immunological parameters

Table 4 indicates a set of immunological parameters for broiler chickens treated with soybean peptides and vitamin E. The highest level ($p \leq 0.05$) of the relative weight of the spleen was obtained in the group supplemented with 5 g of soybean peptides kg^{-1} feed. This result was in agreement with Osho, Xiao, and Adeola (2019), who mentioned a significant increase in spleen weight with increasing concentration of SBP in broiler chickens. The spleen is one of the organs of the immune system in poultry, and its development undoubtedly reflects positively on the immune function in the body.

The soybean peptide groups had higher bursa of Fabricus and bursa index relative weights than the control group, the second treatment (2.5 g SBP kg^{-1}) was noticeably superior ($p \leq 0.05$) on other experimental transactions.

Meanwhile, no significant differences appeared between the control and vitamin E groups In these parameters. In a study conducted by Landy, Kheiri, and Faghani (2020) on the broiler, 6 g kg^{-1} of bioactive peptides derived from cottonseed leads to desirable results in the relative weight of the bursa of Fabricius.

Vitamin E has obvious effects on the fortification of the immunity system (Nayaka, Umakantha, Ruban, Murthy, & Narayanaswamy, 2012), and it increases immunoglobulin in birds (Selim, Gaafar, & El-ballal, 2012). Therefore, the group supplemented with vitamin E was adopted as a positive control group for comparison. As shown in Table 4, the concentration of IgA in serum was significantly increased ($p \leqslant 0.05$) in groups supplemented with 5, 7.5 g SBP kg^{-1}. At the same time. The highest level ($p \leqslant 0.05$) of IgM was obtained in the group supplemented with 5 g of soybean peptides/kg feed as compared with other groups. The findings of the present study simulate those of Osho, Xiao, and Adeola (2019), who demonstrated the positive effect of SBP on the immunity of birds through the significant increase in IgA in plasma when coccidia is challenged. Additionally, there are antibodies in soybean peptides that can enhance the immune function of animals (Hou, Wu, Dai, Wang, & Wu, 2017).

The concentration of IgG in serum was significantly increased ($p \leqslant 0.05$) in groups supplemented with 2.5, 5, 7.5 g SBP kg^{-1} compared with the control and vitamin E group (Table 4). The health of broiler chickens is thought to be improved by IgG. The effect of SBP in boosting immune systems may be a result of the essential amino acids they contain. Glutamate addition in broiler diets has been shown to increase IgG and IgA concentrations as well as growth performance (Chalvon-Demersay et al., 2021). However, a rise in serum's overall antioxidant capability may lead to an increase in antibody levels (Landy, Kheiri, & Faghani, 2021).

Table 4. Effect of experimental treatments on immunological parameters of broilers at 35 days.

Immunological parameters	Control	Soybean bioactive peptide g kg^{-1} diets			Vit. E 250 (mg kg^{-1})	SEM	*P*<
		2.5	5.0	7.5			
Spleen%	0.110^{b}	0.115^{b}	0.181^{a}	0.153^{ab}	0.125^{b}	0.009	0.034
Bursa of fabricus%	0.130^{c}	0.193^{a}	0.163^{b}	0.160^{b}	0.143^{bc}	0.006	0.006
Bursa Index	1.00^{c}	1.49^{a}	1.26^{b}	1.23^{b}	1.10^{bc}	0.048	0.001
IgA (mg mL^{-1})	43.90^{c}	62.28^{ab}	70.88^{a}	70.82^{a}	53.99^{bc}	3.25	0.008
IgM (mg mL^{-1})	217.6^{b}	219.8^{b}	263.3^{a}	249.6^{ab}	219.8^{b}	6.51	0.042
IgG (mg mL^{-1})	98.2^{b}	123.0^{a}	127.6^{a}	142.8^{a}	103.0^{b}	4.95	0.002

SEM = Standard error of mean; $^{a-c}$ = Means within the same raw with different superscripts letters are significantly different ($p \leqslant 0.05$) ; Abbreviations: IgA, immunoglobulin A; IgM, immunoglobulin M; IgG, immunoglobulin G.

Microbacterial count

According to Table 5, the group of birds supplemented with 7.5 g SBP kg^{-1} recorded the lowest number of total bacterial count (TBC) in the ileum, while there were no significant differences between the other experimental treatments. The results recorded the lowest number of these bacteria in the group of supplemented birds with 7.5 g SBP kg^{-1} in caecum.

The lactic acid bacteria (LAB) counts exhibited a significant increase in dietary supplemented with SBP and vitamin E as compared to control in both the ileum and caecum. This bacteria is helping in inhibiting the colonization of pathogenic microflora through their secreted acids and hydrogen peroxide (Dalia, Loh, Sazili, Jahromi, & Samsudin, 2018). The reason for the significant increase in the numbers of The lactic acid bacteria can be attributed to the fact that soybean peptides contain large amounts of nutrients necessary for the growth of beneficial bacteria and increase their number, which leads to an improvement in the balance of the intestinal flora (Kim, Yang, & Kim, 2021).

In the ileum, results showed that there was a significant ($p \leqslant 0.05$) reduction in *Echerichiae coli* count in dietary SBP and vitamin E inclusion diets as compared to the control. While the supplemented groups did not cause any significant differences in E. coli count in the caecum (Table 5). Mohammadrezaei, Navidshad, Gheisari, and Toghyani (2021) reported that feed supplementation with graded levels of cottonseed bioactive peptides decreased the population of *E. coli* in the ileum, and balancing gut microbiota population. Harmful coliform bacteria can degrade the bile salts required for the digestion of fats and thus impede the absorption of fats, and compete with the host for the absorption of nutrients and energy, thus reducing fat, protein and energy efficiency (Dibner & Richards, 2005). It is also possible that the effectiveness of peptides in inhibiting harmful microbes is due to multiple characteristics such as amino acid sequence, size, structure, charge, and electroneutral point (Akalin, 2014).

The reason for increasing the numbers of beneficial bacteria (*Lactobacillus*) and decreasing the numbers of harmful bacteria (*E.coli*) in the group of birds supplemented with SBP could be due to the ability of these peptides to create pores inside the bacterial membranes, and thus inhibit their biosynthetic activities, including weakening the formation of vessels and blocking the gene expression of harmful bacteria to reduce their multiplication (Zaky, Simal-Gandara, Eun, Shim, & Abd El-Aty, 2022). While Aguilar-Toalá, Deering, and Liceaga (2020) found that adding chia protein peptides (<3 kDa) impacted higher antimicrobial activity than chia peptide portion 3–10 kDa. Furthermore, the <3 kDa portion demonstrated an increase in the membrane permeability of E. coli . This demonstrates how soybean peptides' low molecular weight enables them to exert their effects by penetrating deep cellular structures.

Table 5. Micro-bacterial count as affected by supplemented with soybean bioactive peptide and vitamin E (log cfu g^{-1}).

Bacteria types	Control	Soybean bioactive peptide g kg^{-1} diets 2.5	5.0	7.5	Vit. E 250 (mg kg^{-1})	SEM	*P*<
Ileum							
Total bacterial count (TBC) (×10^7)	7.28^{a}	6.02^{ab}	5.86^{ab}	5.64^{b}	6.11^{ab}	0.22	0.05
lactic acid bacteria (LAB) (×10^7)	2.38^{c}	3.52^{ab}	3.80^{a}	3.60^{a}	3.06^{b}	0.14	<0.001
Escherichia coli (*E. coli*) (×10^7)	3.11^{a}	2.47^{b}	2.52^{b}	2.45^{b}	2.49^{b}	0.09	0.05
Caecum							
Total bacterial count (TBC) (×10^7)	8.10^{a}	7.20^{ab}	6.19^{b}	6.78^{ab}	7.14^{ab}	0.23	0.05
lactic acid bacteria (LAB) (×10^7)	3.16^{c}	3.83^{b}	4.23^{a}	3.91^{ab}	3.57^{b}	0.10	<0.001
Escherichia coli (*E. coli*) (×10^7)	3.66	3.28	3.24	3.03	3.17	0.12	0.62

SEM = Standard error of mean; [a-c] = Means within the same raw with different superscripts letters are significantly different (p ≤ 0.05).

Conclusion

From the result of this study, it can be concluded that the bulk of soybean bioactive peptides (68.85 %) are low molecular weight (180-500 Da). Supplementation of SBP (5 and 7.5 g kg^{-1} of diet) can improve Globulin, albumin to globulin ratio, total antioxidant activity, and immune parameters, moreover, the supplementation of SBP led to the reduction of harmful bacteria (*E. coli*) and improved numbers of beneficial (*Lactobacilli*) in the ileum and caecum which can help to enhance intestinal health. On other hand, Vit E (250 mg kg^{-1}) fed diets improved intestinal microflora significantly as compared to control group broiler.

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