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EFFECT OF PROBIOTIC SUPPLEMENT ON NUTRIENT DIGESTIBILITY AND PRODUCTION TRAITS ON BROILER CHICKEN

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ABSTRACT. The aim of the research was to investigate feed nutrient digestion and slaughter indicators of broiler chickens fed a probiotic supplement based on lactic acid bacteria. The experiment lasted for 42 days. Four groups of one-day-old broiler chickens of the Ross-308 cross were selected by the method of analogous groups, each group contained 50 birds. Broilers were kept in group cages considering all zoohygienic requirements. The control group consumed a basic diet (BD), *i.e.*, a complete feed. The experimental groups were additionally fed different doses of a probiotic supplement (by percentage mass of feed). The broiler chickens fed the probiotic supplement had increased digestibility of dry matter, protein, fibre and nitrogen-free extractives (NFE) compared with the control group. The application of probiotic supplement in broiler feeding increases the availability of essential amino acids, *i.e.* lysine, histidine, arginine, threonine, valine, methionine, isoleucine compared with the control. The absorption of Ca, P, Mg, and Mn increased with the probiotic supplement. The probiotic supplement application in the diet of broiler chickens increased the pre-slaughter live weight by 16.7%, the un-gutted body weight by 15.0% and gutted body weight by 17.3%. Probiotic supplement had a positive effect on the digestibility of feed nutrients, increased the absorption of amino acids and minerals in the body broiler chickens.

Introduction

Numerous feed additives such as probiotics, prebiotics, phytobiotics, enzymes, vitamins *etc*. have been used in animal diets in recent years (Park *et al.*, 2014; Mookiah *et al.*, 2014; Anggraeni *et al.*, 2020). However, they do not always have a positive effect on product quality. This issue is important because of advanced technologies for new feed application, the application of chemical and microbiological synthesis products in animal nutrition (Dunkley, 2008; Alavi *et al.*, 2012; Meremäe *et al.*, 2015; Sobolev *et al.*, 2019). Probiotics have become widespread among feed additives of natural origin. They create an unfavourable pH environment for pathogenic and opportunistic microflora and stimulate the growth and biological activity of normal intestinal microflora, having a positive effect on the composition of the microbiocenosis, probiotic microorganisms also produce biologically active substances and amino acids (Liu *et al*., 2012; Salim *et al.*, 2013; Park *et al.*, 2014).

The microbiological industry is actively developing the creation of new and effective feed additives, including probiotic additives based on lactic acid bacteria *(Lactobacillus* and *Enterococcus).* It is known that the hydrolysis of feed nutrients to monomers is carried out using enzymes and acids, and symbiotic microorganisms that are in the digestive tract (Chudak *et al.*, 2020). Some research results have shown the promise of using such probiotic supplements in the diets of farm animals (Dunkley, 2008; Urdzik, 2010; Mookiah *et al.*, 2014; Park *et al.*, 2014; Balukh, 2016; Poberezhets, 2020). However, how the new probiotic supplements that are made according to improved recipes affect the productivity of broiler chickens has not yet been fully studied. Moreover, digestibility of nutrients depends on the species and the animal age, chemical composition,

preparation methods for fee- ding, feeding level and other factors. The aim of this study was to investigate feed nutrient digestion and slaughter indicators of broiler chickens fed a probiotic supplement based on lactic acid bacteria *(Lactobacillus* and *Enterococcus).*

Material and Methods

Formation of groups

The study was carried out on the research farm of Vinnytsia National Agrarian University (Ukraine). The experiment lasted for 42 days, by the method of analogue groups four groups were selected (I – control group, II, III, IV – experimental groups) of one-day broiler chickens "Ross-308" with 50 birds per group, the mean body weight was 62 ± 2 g (Hamungalu *et al.*, 2020).

Ethical statement

The protocol and procedures used in this research were ethical for the animals tested and complied with Directive 2010/63 / EC of the European Parliament.

Description of housing conditions

Broilers were kept in TBB-AV cage batteries (manufactured by VO TECHNA, Kyiv, Ukraine) with a nipple watering system with a stocking density of 20 birds per m^2. The dimensions of the cage were: 1,200 × 1,604 × 408 mm. The cage consisted of flooring, side mesh walls and doors. The floor of the cages was made of a galvanized metal mesh (diameter of the coated wire, 2.2 mm) with holes the size of (16 × 25) mm, which eliminated the possibility of manure soiling, as well as injury to the legs of the bird. Temperatures were as follows: from days 1 to 5 – 32–35 °C, from days 6 to 42 days – 20 °C. Relative humidity 60–70%. Lighting intensity 10–20 lux.

Feed ration and composition

Compound feed TM "Multigain" of joint-stock company "Kyiv-Atlantic-Ukraine" Myronivka, Kyiv region was used. The full-ration compound feeds for broilers PC 5-4 / 7 (Table 1) was used.

The control group consumed a basic diet (BD) as complete feed. The experimental groups were additionally fed different doses (percentage to mass of feed) of a probiotic supplement (Table 2).

Probiotic supplements

Feed additive "Entero-active" is a homogeneous loose mixture without solid lumps coloured from light grey to dark grey colour. The probiotic contains lactic acid bacteria of the genus *Lactobacillus bulgaricus* – 2.0×10^{10} CFU per kg and *Enterococcus faecium* – 2.0×10^{10} CFU per kg. The probiotic "Entero-active", due to the formation of lactic and acetic acids, creates an unfavourable pH environment for pathogenic microflora, stimulates the growth of intestinal normal flora, which has a positive effect on the composition of the microbiocenosis. In addition, probiotic microorganisms produce biologically active substances, enzymes and amino acids.

This feed additive was created in the PE "BTU-centre" Ladyzhyn, Vinnytsia region. The owner of the patent for feed additive "Entero-active" is Vinnytsia National Agrarian University (Podolian *et al.*, 2011). All rights to the results of the study on the impact of Enteroactive on lethal properties belong to Vinnytsia National Agrarian University. The obtained results of scientific research were used during the development of technical conditions: Probiotic "Entero-active" TR (IS) U 15.7-00497236-001: 2012 (Chudak *et al.*, 2012).

Table 1. Composition of compound feed for broiler chickens aged 4–5 weeks

Composition of the diet, %					
Corn	30	Soybean oil	3.0	Vitamin and mineral mixture	1.0
Wheat	27.5	Fodder yeast	3.4	Antioxidant	0.0125
Soybean meal	15.0	Defluorinated phosphate	1.55	Mould inhibitor	0.009
Sunflower meal	12.0	Limestone	1.2	Coccidiostat	0.0097
Fishmeal	5.0	Table salt	0.3	Granule fixer	0.0108
Chemical composition, %					
Crude protein	21.0	Phosphorus	0.7	Linoleic acid	3.21
Crude fiber	5.0	Chlorides	0.307	Sodium	0.2
Methionine + cystine	0.89	Crude fat	6.2	Methionine	0.45
Lysine	1.15	Tryptophan	0.26		
Calcium	0.9	Threonine	0.17		
Vitamins and trace elements, mg kg^{-1}					
Vitamins				Salts	
A	3.00	PP	20.0	Copper	4.8
D3	0.04	E	20.0	Iron	20.0
B1	2.0	K3	2.5	Cobalt	0.48
B24.0B6	2.5	Pantothenic acid	10.0	Zinc	48.0
B12	0.01	Folic acid	0.5	Iodine	0.8
		Biotin	0.05	Selenium	0.28

Table 2. Composition of experimental diets

Group	Duration, days	Feeding characteristics by age, days		
		1–10	11–28	29–42
Control	42	BD (complete feeds)		
II	42	BD + 0.062% Probiotic supplement	BD + 0.025% Probiotic supplement	BD + 0.0125 % Probiotic supplement
III	42	BD + 0.125% Probiotic supplement	BD + 0.05% Probiotic supplement	BD + 0.025% Probiotic supplement
IV	42	BD + 0.25% Probiotic supplement	BD + 0.1% Probiotic supplement	BD + 0.05% Probiotic supplement

The probiotic supplement was fed in the dry form. The preparation and verification of the homogeneity of the mixture of feed and probiotic additives was carried out in the laboratory of Technological Processes of food and processing industry of Vinnytsia National Agrarian University. Mixing took place in the chamber of a vibrating machineVM-5.0 the manufacturer is Ukraine (in the absence of grinding bodies) to homogeneity in the range of 96–98% (Clark *et al.,* 2007).

Measurements and analysis of samples

During the physiological trial, which lasted forfive days, the birds were kept in separate cages. The digestibility of feed nutrients was determined by the difference between their content in the consumed feed and the excreted manure (Kozyr *et al*., 2002). Consumed feed and manure were analysed. Compound feed samples were taken daily for analysis. Feed samples were taken in accordance with DSTU ISO 6497: 2005. Twice a day, morning and evening, manure was collected, which was preserved with toluene and stored in a closed glass container in the refrigerator at +5 °C (Ibatullin *et al.,* 2017). Assessment of morphological and biochemical parameters of the blood were made at the end of the experiments. Four animals were selected by average live weight per group from each group, from which blood was taken in the morning before feeding (Levchenko *et al.,* 2002).The amino acid composition was determined with Automatic Amino acid Analizator (AAA) T-339 (Microtechna, Czech Republic) automatic analyser using LG ANB cation exchange resin with SO_3 active group (Kozyr *et al.,* 2002). Haematological parameters were determined as follows: haemoglobin content – hemoglobin cyanide method using a hemoglobinometer type HG-202 (APEL, Japan), erythrocytes and leukocytes – counted using Goryaev's camera (grid contains 225 large squares; 15 rows of 15 large squares each), to study the feeding efficiency (such as slaughter qualities) of the experimental birds were carried out at the end of the experiment after slaughter – four birds from each group by average live weight per group (Ibatullin *et al.,* 2017).

Slaughter was by cutting the sublingual vein after stunning. Slaughter qualities were investigated according to the following indicators: pre-slaughter live weight of poultry after 12 hours of fasting; mass of ungutted carcass – mass of carcass exsanguinated and without plumage; mass of half-gutted carcass – carcass exsanguinated, without plumage and intestines; the mass of the gutted carcass – the mass of the exsanguinated carcass, without plumage, head, legs, wings, intestine; mass of edible and inedible parts (Ibatullin *et al.,* 2017).

During the experiment, the preservation of livestock was recorded according to the count of dead birds.

Statistical analysis

Processing of experimental data and statistical analysis of the results were performed on a PC using MS Excel 2019 software (Microsoft, USA) and Statistica 12.6 (Dell Technologies, USA) using built-in statistical functions. Statistical functions are functional software modules that implement individual statistical formulas (calculation of average values, correlation coefficient, *etc*.), and can be used in formulas. The small sample method was used. The method of small samples provided for the determination of the arithmetic mean values (x) and the deviation of the arithmetic mean values (± SD). The data in the tables are presented in the form of x ± SD (mean ± standard deviation). Statistical evaluation of differences was performed using Student's t-test. The difference was considered significant if the calculated criterion for the reliability of the difference (experimental) is equal to or exceeds the standard value of the Student's t-test.The results of the average values were considered statistically significant at * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (Rudenko, 2012).

Results

It was found that feed consumption per kg of growth decreased by 7.7% in the III group, and by 12.9% in the IV group (compared to control group). There was no significant effect of the probiotic supplement for group II (Table 3).

Poultry fed additional Entero-active probiotic increased feed conversion in proportion to the dosage of probiotic supplements. However, feed consumption per kg of gain were lower in all of the groups than in the control group.

Table 3. Effect of probiotic supplement on feed consumption, kg

Indicators	Group			
	Control	II	III	IV
Feed consumption during the experiment:				
- total for the group	192.2	189.4	193.8	195.0
- per bird	3.84	3.78	3.88	3.90
Feed consumption per 1 kg gain:				
- total for the group	1.95	1.89	1.80	1.70
- compared (±) to the control group	–	–0.06	0.15	0.25
- compared (%) to the control group	–	3.07	7.7	12.9

The highest digestibilities of protein and nitrogen-free extractives (NFE) were observed when the average dose of the additive was additionally fed; they were higher by 3.4% and 4.0% ($P < 0.001$) than the control. The broilers of the II and IV groups had increased digestibility of protein, although a significant difference with the control was not found (Table 4).

Table 4. Effect of probiotic supplement on coefficients of feed nutrients' digestibility, % ± SD

Traits	Group			
	Control	II	III	IV
Dry matter	77.9 ± 0.39	79.7 ± 1.01	80.3 ± 0.24**	80.2 ± 0.45**
Protein	84.1 ± 0.32	85.6 ± 0.70	87.5 ± 0.11***	84.8 ± 0.95
Fat	94.8 ± 0.08	94.6 ± 0.30	95.0 ± 0.10	93.5 ± 0.22**
Fibre	6.1 ± 2.05	26.3 ± 3.43**	22.1 ± 1.14***	37.2 ± 2.26***
NFE	86.6 ± 0.24	88.1 ± 0.57*	90.6 ± 0.16***	87.9 ± 0.17**

* significant at $P < 0.05$ compared with control group; ** significant at $P < 0.01$ compared with control group; *** significant at $P < 0.001$ compared with control group. NFE – nitrogen-free extractives

Feeding the probiotic maximum dose increased the dry matter digestibility ($P < 0.01$), the average dose also increased the dry matter digestibility ($P < 0.01$) relative to the control group. The maximum dose of the additive caused the highest digestibility of fibre by broilers ($P < 0.001$). However, in groups II and III the fibre digestibility was higher than in the control by 20.2% ($P < 0.01$) and 16.0 % ($P < 0.001$), respectively. Group II consumed the lowest dose of probiotic supplement; they outperformed NFE digestibility in the control group by 1.5% ($P < 0.05$). The consumption of probiotics in group IV increased NFE digestibility ($P < 0.01$). However, it caused a decrease of fat digestibility ($P < 0.01$). Probiotics application for broiler chicken feeding had a positive effect on the digestibility of amino acids (Table 5). The broiler chickens fed the average dose of the supplement had the highest digestibility of amino acids. The digestibility of such essential amino acids as lysine, histidine, arginine, valine, methionine, isoleucine and leucine in group III significantly exceeded the control group values ($P < 0.001$). In group II the absorption of phenylalanine was lower ($P < 0.001$) than the control sample.

Feeding the minimum dose of probiotics caused a decrease in the coefficients of digestion of amino acids compared to control values. The highest digestibility of aspartic and glutamic acids was found in group III, which was higher ($P < 0.001$) compared to the control group. The digestibilities of threonine ($P < 0.001$), serine ($P < 0.001$), proline ($P < 0.001$), glycine ($P < 0.001$), alanine ($P < 0.001$) and cystine ($P < 0.001$) were higher in group III compared to the control. The highest content of tyrosine was observed in group IV ($P < 0.01$). The retention of mineral elements in the feed of broiler chickens are listed (Table 6).

Table 5. Effect of probiotic supplement on digestibility of amino acids by broiler chickens, % ± SD

Amino acids	Group			
	Control	II	III	IV
Lysine	87.0 ± 0.24	84.3 ± 0.74*	91.8 ± 0.15***	88.5 ± 0.21**
Histidine	90.5 ± 0.22	87.7 ± 0.49**	94.3 ± 0.09***	91.8 ± 0.45*
Arginine	88.1 ± 0.09	82.7 ± 0.90**	93.0 ± 0.21***	91.4 ± 0.24***
Aspartic acid	83.4 ± 1.52	79.0 ± 1.52*	88.7 ± 0.14***	86.0 ± 0.30**
Threonine	81.9 ± 0.29	76.1 ± 0.93**	89.4 ± 0.29***	85.5 ± 0.28***
Serine	82.8 ± 0.49	72.9 ± 1.25***	88.8 ± 0.19***	85.8 ± 0.40**
Glutamic acid	83.9 ± 0.34	86.2 ± 0.65*	93.6 ± 0.12***	90.9 ± 0.21***
Proline	85.9 ± 0.334	79.5 ± 0.53***	90.2 ± 0.10***	88.2 ± 0.21**
Glycine	76.9 ± 0.44	61.1 ± 1.88***	83.2 ± 0.45***	78.1 ± 0.42
Alanine	76.1 ± 0.75	58.4 ± 2.29***	82.7 ± 0.48***	74.5 ± 0.43
Cystine	89.8 ± 0.10	85.2 ± 1.16**	92.2 ± 0.19***	88.3 ± 0.55
Valine	84.5 ± 0.35	71.4 ± 1.49***	88.6 ± 0.36***	86.2 ± 0.43*
Methionine	93.9 ± 0.07	92.9 ± 0.43	96.5 ± 0.40***	92.9 ± 0.28*
Isoleucine	78.4 ± 0.29	75.0 ± 0.95*	86.0 ± 0.30***	82.1 ± 0.35***
Leucine	85.8 ± 0.26	75.7 ± 0.81***	89.9 ± 0.11***	87.9 ± 0.23***
Tyrosine	86.6 ± 0.49	75.2 ± 0.85***	88.7 ± 0.51*	91.2 ± 0.60**
Phenylalanine	88.9 ± 0.45	57.0 ± 1.30***	89.5 ± 0.23	88.0 ± 0.28

* significant at $P < 0.05$ compared with control group; ** significant at $P < 0.01$ compared with control group; *** significant at $P < 0.001$ compared with control group.

Table 6. Effect of probiotic supplement on retention of mineral feed elements, % ± SD

Mineral elements	Group			
	Control	II	III	IV
Calcium	17.9 ± 1.53	39.9 ± 2.82***	40.1 ± 0.91***	41.4 ± 1.49***
Phosphorus	60.8 ± 1.42	67.7 ± 1.95*	66.8 ± 1.02*	66.4 ± 2.18
Magnesium	31.8 ± 1.14	32.7 ± 3.95	40.8 ± 0.68***	44.0 ± 1.69**
Manganese	7.2 ± 2.45	31.8 ± 3.29**	26.0 ± 1.13***	40.3 ± 1.36***

* significant at $P < 0.05$ compared with control group; ** significant at $P < 0.01$ compared with control group; *** significant at $P < 0.001$ compared with control group.

Broilers additionally fed probiotic additive had increased retention of Ca and Mn in the all treatment groups ($P < 0.001$ and $P < 0.001$) compared to the control group.

A significant difference in increase in the phosphorus absorption was observed in groups II and III ($P < 0.05$) compared to the control group. The probiotic supplement had a positive effect on Mg content in groups III and IV ($P < 0.001$) compared to the control.

The effect of probiotic supplement on morphological analysis of the blood in broilers are presented in Table 7.

Table 7. Effect of probiotic supplement on morphological parameters of broiler blood

Parameters	Group			
	Control	II	III	IV
Leukocytes, G l^{-1}	18.1 ± 0.96	20.8 ± 0.92	20.1 ± 0.80	21.0 ± 1.88
Erythrocytes, T l^{-1}	3.0 ± 0.17	2.9 ± 0.08	2.8 ± 0.07	2.8 ± 0.04
Haemoglobin, g l^{-1}	106.5 ± 5.28	122.0 ± 4.97	121.5 ± 2.60*	116.0 ± 2.49
ESR, mm h^{-1}	1.7 ± 0.55	1.5 ± 0.33	1.7 ± 0.55	1.5 ± 0.33

* results of the mean values ± SD were considered significant at $P < 0.05$ compared with control group. ESR – erythrocyte sedimentation rate

The highest number of leukocytes relative to the control group was recorded in group IV, it was higher by 16.0%, but no significant difference was found. The erythrocyte level of the treatment groups did not differ, neither was the erythrocyte sedimentation rate (ESR) affected by the experimental diets. The highest haemoglobin contents were observed in groups II and III ($P < 0.05$). The probiotic additive had a positive effect on the slaughter indicators of broiler chickens (Table 8).

Broilers in groups III and IV had a higher pre-slaughter live weight ($P < 0.001$) compared to the control and group II. The broilers in groups III and IV had an increased weight of ungutted and gutted carcasses compared with chickens in the control group and group II. The weight of semi-gutted carcass was not affected by the probiotic supplementation.

Table 8. Effect of probiotic supplement on slaughter qualities indicators ± SD of broiler chickens

Indicators	Group			
	Control	II	III	IV
Pre-slaughter weight, g	2 064.5 ± 15.3	2 104.0 ± 14.8	2 258.0 ± 26.7***	2 410.7 ± 95.6**
Ungutted carcass weight, g	1 867.2 ± 8.3	1 923.0 ± 55.9	2 031.0 ± 24.8***	2 148.0 ± 93.1**
Semi gutted carcass weight, g	1 650.5 ± 53.0	1 652.5 ± 73.0	1 793.2 ± 30.0	1 918.0 ± 101.6
Gutted carcass weight, g	1 406.0 ± 18.2	1 412.5 ± 89.5	1 540.5 ± 29.5**	1 650.0 ± 82.14*
Mortality of broiler chickens, %	4.0	3.0	2.5	2.0

* significant at $P < 0.05$ compared with control group; ** significant at $P < 0.01$ compared with control group; *** significant at $P < 0.001$ compared with control group.

Discussion

This research showed that probiotic supplement application caused a reduction of feed consumption and increased broiler productivity. The results are consistent with the study by Podolian (2016) showing the effective action of a probiotic feed additive on live weight, the growth and slaughter indices of cross Ross-308 broiler chickens. The feed consumption per kg of growth was higher in the control group chickens. However, it was found that the administration of probiotic feed additive increased the live weight, the undressed carcass weight, half-dressed carcass weight and dressed carcass weights compared to the control group. Cengiz e*t al*. (2015) have also reported a positive effect on the productivity and growth of broilers fed probiotic additives.

It is conjectured that the better slaughter rates of broilers fed probiotic supplement was caused by increased feed intake and improved digestibility of feed nutrients, amino acids and minerals (Rajesh *et al*., 2020). According to He *et al*. (2019), the probiotic is able to improve the activity of digestive enzymes of poultry. Slaughter rates of broilers of groups III and IV increased, in particular preslaughter live weight and gutted carcasses live weight.

The results are consistent with research results that were carried out with other broilers. The positive effect of probiotics on slaughter rates has been previously mentioned, in particular, that their use increases slaughter qualities and improves the development of internal organs and digestive organs (Patreva, Shevchenko, 2010; Otchenashko, 2012). The addition of probiotics (500 mg/kg in the first phase and 300 mg/kg in the second phase) could improve broilers' growth performance, nutrient retention, and serum antioxidant capacity, improve their intestinal health *via* improving jejunal mucosal barrier function and intestinal morphology.

Previous studies (Urdzik, 2010; Balukh, 2016) confirm the positive effect probiotic additives have on amino acid absorption and retention of mineral feed elements in poultry. The findings on increased nutrient digestibility under the influence of probiotic additives are consistent with previous studies (Belova *et al*., 2009; Urdzik, 2010; Fedorchenko, 2017; Azemraw, Sewalem, 2017).

Haemoglobin increased in the blood of broiler chickens that consumed a probiotic during the experiment. The results are consistent with studies that have shown a positive effect of probiotic feed additives on the animals' haemaglobin (Mashkin, 2010; Poberezhets, 2020).

The positive effect of probiotics on slaughter yield has been noted previously (Patreva, Shevchenko, 2010; Otchenashko, 2012) in particular, that their use increases slaughter live, weight of ungutted and gutted carcasses.

Translocation of probiotic bacteria from the intestine to the blood and the following bacteraemia is one of the critical issues that should be considered when probiotics are supplemented in the diet (Lopetuso *et al*., 2017). According to Zaghari *et al*. (2020), probiotic bacteria do not enter the bloodstream following use of a probiotic feed additive for poultry feeding. So there is no likelihood of complications from high microbial count and septicaemia arising from the presence of these bacteria and bacteriocin produced by them in the blood of broiler chickens. Thus, probiotic feed additives have a positive effect on the productivity, digestibility of nutrients in broiler feed and are safe for poultry.

Conclusion

This study has shown the use probiotic supplement had a positive effect on the digestibility of feed nutrients, increased the absorption of amino acids and minerals in the body and enhanced metabolic processes in broiler chickens. In addition, the feed consumption per kg of growth was lower when probiotic supplement was used. Additionally, the pre-slaughter live weight increased and the weight of ungutted carcasses and gutted carcasses also increased.

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Conflict of interest
The author declares that there is no conflict of interest regarding the publication of this paper.

Author contributions
JP – study conception and design;
JP, VY, VR, IK – acquisition of data;
JP, RC – analysis and interpretation of data;
IK, JP – drafting of the manuscript;
IK – editing the manuscript;
RC, JP – critical revision and approval of the final manuscript.

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