Heavy metals, nitrates and radionuclides in milk of cows depending on their stress resistance

O. M. Chernenko*, R. A. Sanzhora*, N. M. Shulzhenko*, R. V. Mylostyyvi*, O. V. Denisyk**

*Dnipro State Agrarian and Economic University, Dnipro, Ukraine
**State Institute of Grain Culture NAAS of Ukraine, Dnipro, Ukraine

Introduction

Heavy metals are recognized as pathogenic pollutants of atmospheric air, water bodies and soils at global and regional scales (Kumar et al., 2019). This requires constant monitoring of their content and taking measures to neutralize these hazardous components (Sikdar & Kundu, 2017). However, not all heavy metals are equally hazardous. Some of them are microelements necessary for the regular functioning of living organisms. However, when present in excess they have a negative impact on the growth, development and reproductive functions of microorganisms, plants, animals and humans (Henkel, 2018). Lead, cadmium, copper, zinc are highly toxic heavy metals penetrating into soils with organisms, plants, animals and humans. However, when present in excess they have a negative impact on the state of pressure which occurs in the body under the influence of stress impairs homeostasis. It can affect the quantity and quality of products, can cause disorders in the metabolic processes, the synthesis of milk components and introduction of some harmful substances into milk, which are differently accumulated in the body of cows with diverse reactions to stress and can be excreted with milk in different levels. We established that different resistance of cows to stress can affect the concentration of the investigated harmful substances in milk. Concentration of lead, cadmium, zinc and nitrates was lower in milk of more stress-resistant cows (the first group). Their milk had a lower specific activity of $^{137}$Cs and $^{90}$Sr. For all the seasons, the reliable results were obtained only for nitrates, and $^{137}$Cs and $^{90}$Sr – in spring, summer and autumn. Thus, milk of cows with higher resistance to stress is safer in terms of contents of the investigated elements. We have drawn a conclusion that stress can affect the ability of the body to excrete via the milk harmful elements which enter the body with food and water. Perspectives of further research are determining the effect of cows' stress resistance on the suitability of milk for the production of baby food products.

Keywords: milk contaminants; elimination of harmful substances; milk safety; season of the year; cortisol; cattle.

The paper presents the results of studies on the content of cesium, strontium, zinc, cadmium, copper, lead and nitrates in the milk of Ukrainian black-and-white milk breed cows with different resistance to stress. The resistance of cows to stress was determined by concentration of cortisol in the blood one hour after a stress effect. The following factors were stressful: fixation of animals for one hour and pre-selection of blood by a veterinarian for general analysis. The objective of the research was establishing a connection between the different adaptive potential of Ukrainian black-and-white milk breeds and the ability of their bodies to excrete via the milk heavy metal salts, nitrates and radioactive elements which enter the body with food and water. The scientific hypothesis was that the state of pressure which occurs in the body under the influence of stress impairs homeostasis. It can affect the quantity and quality of products, it can cause disorders in the metabolic processes, the synthesis of milk components and introduction of some harmful substances into milk, which are differently accumulated in the body of cows with diverse reactions to stress and can be excreted with milk in different levels. We established that different resistance of cows to stress can affect the concentration of the investigated harmful substances in milk. Concentration of lead, cadmium, zinc and nitrates was lower in milk of more stress-resistant cows (the first group). Their milk had a lower specific activity of $^{137}$Cs and $^{90}$Sr. For all the seasons, the reliable results were obtained only for nitrates, and $^{137}$Cs and $^{90}$Sr – in spring, summer and autumn. Thus, milk of cows with higher resistance to stress is safer in terms of contents of the investigated elements. We have drawn a conclusion that stress can affect the ability of the body to excrete via the milk harmful elements which enter the body with food and water. Perspectives of further research are determining the effect of cows' stress resistance on the suitability of milk for the production of baby food products.
Radioactive elements are undeniable mutagens. The main ones that determine the radiation state are $^{60}$Sr and $^{137}$Cs (Omar-Nazir et al., 2018). The chemical properties of cesium and strontium radionuclides are similar to those of potassium and calcium; therefore, they are easily included in the trophic chain “soil → plants → plant production” and accumulate in food products (Gudkov, 2006). Most of the territory of Ukraine is contaminated with $^{137}$Cs which emerges when uranium and plutonium decay. $^{137}$Cs forms highly soluble compounds, is available to plants and accumulates in them (Burger & Lichtscheidl, 2018). The inflow of radioactive elements and heavy metals into the human body with food is mainly due to their transition from soil to plants and then to livestock products (Sugiyama et al., 2007; Burger & Lichtscheidl, 2018).

The ability to adapt to the effects of environmental conditions, while preserving homeostasis, is an essential property of all living organisms. Throughout life a constant adaptation to changes of these conditions occurs. On farms, animals are exposed to various factors that can cause stress: the transfer of animals from one group to another, fighting for dominance, transportation, veterinary care, living in large groups, changes in technology, the impact of harmful substances on the organism and others (Mylstovyi & Chernenko, 2019). Under these conditions, the organism can fall out of state of equilibrium with the environment (McBerg et al., 2000; Carroll et al., 2013), and there are some homeostasis disorders (Fried, 1989; Pigorn et al., 2008). Due to the individual characteristics of the organism some animals are less susceptible to daily operational loads and react in a relatively balanced manner, while others have a sharp reaction which unbalances the neuro-hormonal system and leads to the disorders in homeostasis (Leijcourt et al., 1982; Borell et al., 2007a). Researchers aimed to determine the individual adaptive abilities of animals in response to stress by measuring the dynamics of cortisol concentration in the blood before and after one hour after the stress effect (Hopster et al., 1999). It was determined that the initial concentration of cortisol in the blood of cows was 0.5–1.50 ng/mL (1.4–41.4 nmol/L), and after the repeat sampling of blood 4.5–22.6 ng/mL (12.4–62.3 nmol/L). Attention was paid to the fact that the maximum concentration of cortisol in the blood occurred 30 and 60 minutes after the impact of stress. Some animals resistant to stress restore homeostasis faster. They do not undergo a sharp deterioration of health, do not decrease performance and the reproduction function is not disrupted (Beithart et al., 1982; Borell et al., 2007b). Less stress-resistant animals react to the same stress factor so severely that it causes abrupt changes in the organism (Gieszck et al., 1985; Wolfson et al., 2000). They may have dysfunction of the udder during machine milking, decrease in milk yield and the quality of milk and shortening of the life of cows in herds, since stress is a mechanism for body deterioration (Wenzel et al., 2003; Heikkilä et al., 2012). At the same time, no evidence is available on how disorder of homeostasis and general stress resistance affect the ability of the body of cows to remove harmful substances with milk, although the safety of the milk depends on it. The study of local bovine animals represents a scientific novelty. Their ability to excrete harmful substances with milk due to stress is still a problem that needs to be solved. However, this issue is relevant since industrial pollution of the environment in Ukraine is increasing. In particular, a lot of industrial plants which are the source of contamination are concentrated in Dnipropetrovsk region: an unmodernized thermal power plant, a car battery manufacturing plant, the Piatykhatky ore mining complex, and in Taromtske village near Dnipro city radioactive wastes are stored. In addition, the Zaporizhzhya Nuclear Power Plant is a mining complex, and in Taromtske village near Dnipro city radioactive wastes are stored. In addition, the Zaporizhzhya Nuclear Power Plant is a nuclear facility which has spent a significant amount of time on reactor operations. This situation is particularly dangerous due to the proximity of the nuclear power plant to populated areas.

To determine the level of cortisol, test kits of the company Alkar-Bio (Russia) and IFA reader Labline 022 Labline company (Austria) were used. The method is based on the competition between the unlabeled antibody and the enzyme-labeled antigen for a certain number of antibody bonds. The number of enzyme-labeled antigens bound to the antibodies is inversely proportional to the concentration of the unlabeled antigens. The dairy productivity of the cows was taken into account (Table 1).

### Table 1

| Milk productivity of cows for the first lactation (x ± SD) |
|----------------------------------------------------------|
| Features                                                 | Groups of animals |
|----------------------------------------------------------|
| Annual yield for 305 days, kg                             | L, n = 11          |
|                                                        | II, n = 51         |
|                                                        | III, n = 11        |
| An in % of fat                                          | 5286 ± 395$^{a}$   |
|                                                        | 4619 ± 462$^{a}$   |
|                                                        | 4110 ± 358$^{a}$   |
| Content in milk: fat, %                                   | 3.66 ± 0.09$^{a}$  |
|                                                        | 3.62 ± 0.24$^{a}$  |
|                                                        | 3.61 ± 0.32$^{a}$  |
| Protein, %                                               | 3.18 ± 0.008$^{a}$ |
|                                                        | 3.09 ± 0.16$^{a}$  |
|                                                        | 3.06 ± 0.14$^{a}$  |

Note: different letters indicate values which reliably differed from another within the table according to the Bonferroni correction.

The data presented in Table 1 indicate that the content of fat and protein in the milk of the animals groups differed little. However, milk yields were higher by 1176 kg (P = 54.2, F_{(0.05)} = 4.35, P = 4.1 • 10$^{-7}$) in cows of the first group, by 509 kg (P = 11.8, F_{(0.05)} = 4.00, P = 1.1 • 10$^{-7}$) in cows of the second groups, compared with the one year old cows of the third group.

### Materials and methods

The experimental part of the research was carried out in accordance with the requirements of European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, 1986). The resistance of cows to stress was determined by the concentration of cortisol in the blood one hour after the stress effect (Hopster et al., 1999). The following factors were stressful: fixation of animals for one hour and pre-selection of blood for general analysis. Blood samples were taken by a veterinary surgeon, from the left jugular vein. Blood was taken in the morning before feeding the animals. Immediately after labeling, each test tube was placed in a container with ice, in which it was transported to the laboratory of “VIS-Medik” plc (Ukraine, Dnipro). Cows were divided into groups with high, medium and low resistance to stress, respectively, with the deviation: the first group was less than x – SD, the second was from x – SD to x + SD, the third group was more than x + SD. The concentration of cortisol on average was 158.9 nmol/L for 73 experimental cows, and a standard deviation of 54.3 nmol/L. Experimental cows (n = 73) had cortisol concentrations in blood serum after stress in the range 20–283 nmol/L. Cows with a high resistance to stress had (I group, n = 11) the concentration equating 20–100, cows of the middle group had the concentration (II, n = 51) 106–213 and the concentration in the cows with low stress resistance (group III, n = 11) was 219–283 nmol/L (Fig. 1).

![Fig. 1. Cortisol distribution frequency one hour after stress effect: n = 73; x = 161; SD = 52; max = 283; min = 20](image-url)
A chemical, toxicological and radiological analysis of fodders, water and milk from highly resistant, medium and low-stressed groups of cows was carried out depending on the seasons of the year in Dnipropetrovsk Regional State Laboratory of Veterinary Medicine.

The salts of heavy metals were determined on a spectrophotometer Helios Delta, Thermo Electron company (USA). Nitrates were determined in milk and fodder on the ionometer Cyber Scan Ion 510 Eutech company (Netherlands). The content of the radioactive elements, $^{137}$Cs and $^{90}$Sr, was measured using universal spectrometric complex Gamma plus Exim company (Ukraine).

The number of harmful substances which penetrated the organism during the year was determined by calculation, based on their content in the seasonal fodders in diets per day. The amount of fodder consumed per day (in kg) was multiplied by the number of harmful substances in 1 kg of this fodder; the result was multiplied by the duration of the corresponding season of the year. According to the general consumption of 1 kg of this fodder, the result was multiplied by the duration of the corresponding season of the year. According to the general consumption of 1 kg of this fodder, the result was multiplied by the duration of the corresponding season of the year. According to the general consumption of 1 kg of this fodder, the result was multiplied by the duration of the corresponding season of the year.

Analysis of the data was performed using Statistics 6.0 (StatSoft Inc., USA) program. The data are presented in tables as $x \pm SD$ ($x \pm SD$).

Results

The largest amount of lead in milk of experimental cows was observed in summer. In particular, the cows of the first group had the lowest lead content in comparison with the third group by 3 μg/kg ($F = 45.00, F_{0.016} = 4.35; P = 1.6 \times 10^{-4}$) in the spring and 2 μg/kg ($F = 13.8, F_{0.016} = 4.35; P = 1.3 \times 10^{-4}$) in winter. Cows of the second group took an intermediate position.

Table 2

| Seasons | Groups of animals |
|---------|------------------|
|         | I                | II               | III               |
|         | Total, μg/kg     | Winter, μg/kg    | Summer, μg/kg     | Autumn, μg/kg  |
| Spring  | 10.1 ± 1.4$^a$   | 11.7 ± 0.8$^a$  | 13.1 ± 1.1$^a$   |
| Summer  | 41.2 ± 4.3$^a$   | 42.4 ± 3.7$^a$  | 45.2 ± 4.9$^a$   |
| Autumn  | 13.6 ± 1.8$^a$   | 14.1 ± 1.6$^a$  | 15.2 ± 1.9$^a$   |
| Winter  | 14.1 ± 1.2$^a$   | 14.6 ± 1.4$^a$  | 16.1 ± 1.4$^a$   |

The content of lead (μg/kg) in milk of cows with different reactions to stress (x ± SD, n = 11, duration of experiment – 365 days)

Note: see Table 1.

Table 3

The content of cadmium (μg/kg) in milk of cows with different reactions to stress (x ± SD, n = 11, duration of experiment – 365 days)

| Seasons | Groups of animals |
|---------|------------------|
|         | I                | II               | III               |
|         | Total, μg/kg     | Winter, μg/kg    | Summer, μg/kg     | Autumn, μg/kg  |
| Spring  | 2.12 ± 0.42$^a$  | 1.53 ± 0.51$^a$ | 3.12 ± 1.2$^a$   |
| Summer  | 2.42 ± 0.23$^a$  | 2.11 ± 0.64$^a$ | 2.34 ± 0.71$^a$  |
| Autumn  | 1.84 ± 0.44$^a$  | 2.51 ± 0.32$^a$ | 2.45 ± 0.52$^a$  |
| Winter  | 1.91 ± 0.52$^a$  | 2.12 ± 0.44$^a$ | 3.11 ± 0.61$^a$  |

Note: see Table 1.

Table 4

The content of zinc (μg/kg) in milk of cows with different reactions to stress (x ± SD, n = 11, duration of experiment – 365 days)

The content of cadmium in milk of cows by season of the year differed depending on their reaction to stress, except for the summer, when there was a stable level of it with no changes. Cows of the first and second groups had the lowest amount of cadmium in spring and the third group – in summer. In the third group of cows, the milk content of this element was the largest, compared with the first group, by 1.91 μg/kg ($F = 31.2, F_{0.016} = 4.35; P = 1.8 \times 10^{-3}$) in spring and 1.20 μg/kg ($F = 27.3, F_{0.016} = 4.35; P = 4.1 \times 10^{-3}$) in winter. The cows of the second group took an intermediate position.

Table 5

The content of copper (μg/kg) in the milk of cows with different reactions to stress (x ± SD, n = 11, duration of experiment – 365 days)

Note: see Table 1.

The lowest level of copper in the milk of animals of all groups was observed in summer, the highest in autumn. Regardless of the season, the content of copper was stably higher in the third group of cows. The difference between the first and the third groups was 29 μg/kg in spring, 16 μg/kg in summer, 8 μg/kg in autumn and 6 μg/kg in winter (the result is unreliable). The cows of the second group took an intermediate position.

Table 6

The content of zinc (μg/kg) in the milk of cows with different reactions to stress (x ± SD, n = 11, duration of experiment – 365 days)

Note: see Table 1.

The content of zinc also varied depending on the season. In all groups, its lowest level was observed in spring, the highest in autumn. In all seasons the lowest level of zinc was in milk of cows of the first group. The largest differences were observed in the spring when the difference between the first and the third group was – 184 μg/kg ($F = 29.1, F_{0.016} = 4.35; P = 2.8 \times 10^{-3}$), and between the first and the second group – 92 μg/kg ($F = 7.6, F_{0.016} = 4.35; P = 1.2 \times 10^{-2}$) while the third and the second group differed by 92 μg/kg ($F = 8.5, F_{0.016} = 4.35; P = 8.6 \times 10^{-3}$).

Table 7

The content of nitrates (μg/kg) in the milk of cows with different reactions to stress (x ± SD, n = 11, duration of experiment – 365 days)

Note: see Table 1.

Increase in the content of nitrates was observed in summer and autumn in animals of all groups. However, a dependence of nitrate content on cows‘ stress resistance group was revealed. In particular, the difference between the first and third groups was 87 μg/kg ($F = 51.9, F_{0.016} = 4.35; P = 5.6 \times 10^{-3}$) in spring, 609 μg/kg ($F = 75.5, F_{0.016} = 4.35; P = 3.2 \times 10^{-3}$) in summer, 51 μg/kg ($F = 81.7, F_{0.016} = 4.35; P = 1.7 \times 10^{-3}$) in autumn, 135 μg/kg ($F = 48.8, F_{0.016} = 4.35; P = 8.9 \times 10^{-3}$) in winter. The cows of the second group took an intermediate position.

In addition to these elements, the specific activity of cesium and strontium in the milk of cows of different groups was determined.
Seasons | Groups of animals | I | II | III
--- | --- | --- | --- | ---
Spring | 3.24 ± 0.13 | 3.61 ± 0.15 | 3.91 ± 0.21 |
Summer | 3.51 ± 0.18 | 3.68 ± 0.31 | 3.92 ± 0.14 |
Autumn | 2.95 ± 0.19 | 3.47 ± 0.25 | 3.91 ± 0.35 |
Winter | 5.89 ± 0.16 | 5.92 ± 0.17 | 6.11 ± 0.24 |

Note: see Table 1.

Table 9
Specific activity of strontium (Bq/kg) in milk of cows with different reactions to stress (x ± SD, n = 11, duration of experiment – 365 days)

| Seasons | Groups of animals | I | II | III |
--- | --- | --- | --- | ---
Spring | 2.53 ± 0.19 | 2.69 ± 0.15 | 2.98 ± 0.17 |
Summer | 2.33 ± 0.21 | 2.61 ± 0.19 | 2.95 ± 0.23 |
Autumn | 2.04 ± 0.14 | 2.38 ± 0.13 | 2.91 ± 0.17 |
Winter | 2.81 ± 0.31 | 2.84 ± 0.21 | 2.86 ± 0.27 |

Note: see Table 1.

The seasonal fluctuations in the content of harmful substances in the milk of cows of different groups are explained by the same fact. In summer, when the weather is extremely hot, experimental cows are put out to pasture and grazed on meadows without sheds. Animals do not have the opportunity to shelter from the sun under the canopies, because they are not even provided by construction projects. After all, earlier, there was no problem of sunburn, except in the South of the country. This can cause stress (Bernabucci et al., 2014; Schüller et al., 2014). The results of the research on this issue in the same agricultural firm have already been published (Chemenko et al., 2018). As a consequence, a decrease in milk yield can occur due to the deterioration of feed consumption among all animals, but to a greater extent among cows with low resistance to stress. They return from summer pasture to confined premises. Regrouping and adaptation to the altered technological conditions can also lead to a decrease in milk yield, particularly in low stress-resistant animals. However, milk productivity was investigated and it was found that milk yields were dramatically different. Cows with higher resistance to stress had higher milk yields. They can excrete more lead, cadmium, copper, zinc, nitrates, cesium and strontium to a lesser extent. The cows of the second group took an intermediate position.

Discussion
Over the past 30 years, scientists have been actively studying the influence of heavy metals on the environment, health of animals and humans (Duxbury, 1985; Ali et al., 2013). Very few positive changes have occurred in the environmental conditions of Ukraine. Therefore, the study of the safety of cow’s milk is of particular relevance (Boyko et al., 2016). Pollution of the environment occurs due to atmospheric emissions of enterprises (Hiatt & Huff, 1975; Chaney et al., 2000), waste from livestock farms and the use of mineral fertilizers and pesticides on agricultural land. With the use of organic materials, the concentration in the soil of such chemical elements as lead, cadmium, copper, zinc, iron, manganese increases (Dudka et al., 1996), and therefore the risk of them entering into the body of both animals and humans increases. Heavy metals are a group of microelements, i.e. metals and metalloids with a greater atomic density than 4.1 g/cm³, for example, Cu, Zn, Hg, Cd, Pb, Sn, Fe, Mn, Ag, Cr, Co, Ni, As, Al and others. The ions of these metals are considered to be the most common toxic mineral pollutants of soil and water systems (Mohammed et al., 2011). Microelements in soils may be in water-soluble form, available to plants. Carbonates, oxides and organic compounds are less available. The ratio of these fractions very affects the metabolic processes of the body. It enters the environment from the casting manufacturing, combustion of industrial waste, coal, oil, melting and processing of non-ferrous metals or from exhaust gasses of motor vehicles. Cadmium is a heavy metal that is widespread in various ecosystems, including soils and ore, where it is associated with zinc. It is present in low concentrations (Shirai et al., 1993). It becomes introduced into the environment from volcanic eruptions and by release from plants (Williams & Harrison, 1984). Cadmium is a mutagen, a carcinogen, a potential genetic hazard, cumulative, highly toxic in the organisms of animals and humans and blocks the functioning of enzymes vital for the organism. Copper is widely used in various sectors of the economy, including rural areas. Zinc, similarly to copper, is a microelement that is vital for the growth and development of any organism. It is present in all the organs and tissues of animals. The level of nitrates requires constant control, since they have a wide range of toxic effects, affecting the body at different biological levels. The hypoxic state can mostly occur in such tissues of the body, where an intense cell division is taking place (Kabiraya & Ramani, 2018). Accumulation of harmful substances in the soil-plant system affects a number of soil properties (Wong, 2003). For example, with growing acidity of the soil, their mobility in its layers and the introduction of $^{137}Cs$ into plants increase (Howard et al., 2017, Abraham et al., 2018). According to the Institute of Agriculture of Polissya National Academy of Sciences of Ukraine, among the studied grain crops, oats accumulated $^{137}Cs$ most of all, winter wheat, spring barley and finally winter rye accumulated the least amount of $^{137}Cs$ (Deisan et al., 2009). Leguminous cultures with high calcium content accumulate more strontium compared to cereals (Dubchak, 2017).

Research has revealed evidence that variations in adaptive capabili-ty of cows affect their ability to excrete harmful substances with milk. The source of introduction of lead, cadmium, copper, zinc, nitrates, cesium, and strontium into the organism of animals is food and water (Dubchak, 2017; Abraham et al., 2018). Then these substances enter the bloodstream. More productive cows naturally consume more water and feed and have a higher conversion rate. There is no special mechanism in the body for the excretion of these substances except for urine, and lactating animals can excrete them with milk. Research has demonstrated that stress under the influence of adrenaline increases arterial pressure (Friend, 1980). This provides more intense filtration of these substances from the blood vessels. Because of this, the kidneys of animals which are more sensitive to stress may excrete more harmful substances with urine, as well as with milk. The difference in the excretion of these substances in cows of different groups with urine was not investigated. However, milk productivity was investigated and it was found that milk yields were dramatically different. Cows with higher resistance to stress had higher milk yields. They can excrete more lead, cadmium, copper, zinc, nitrates, cesium and strontium in absolute quantities with milk. However, when daily milk yields are higher the concentration of these substances per 1.0 L of milk may be less in comparison with animals with lower level of milk yields.

The seasonal fluctuations in the content of harmful substances in the milk of cows of different groups are explained by the same fact. In summer, when the weather is extremely hot, experimental cows are put out to pasture and grazed on meadows without sheds. Animals do not have the opportunity to shelter from the sun under the canopies, because they are not even provided by construction projects. All these conditions are more extreme in the South of the country. This can cause stress (Bernabucci et al., 2014; Schüller et al., 2014). The results of the research on this issue in the same agricultural firm have already been published (Chemenko et al., 2018). As a consequence, a decrease in milk yield can occur due to the deterioration of feed consumption among all animals, but to a greater extent among cows with low resistance to stress. However, milk productivity was investigated and it was found that milk yields were dramatically different.
may include the influence of stress resistance of cows on the suitability of milk to be used in production of baby food products.

Conclusions

Out of the studied elements introduced into the organisms of cows with water and fodder during a year, those found in the highest concentrations were nitrate, zinc and copper. The concentrations of lead, cadmium, zinc and nitrate were lower in the milk of cows from the first group. It had the lowest specific activity of $^{137}$Cs and $^{90}$Sr. Reliable results for all seasons were obtained only for nitrate and for $^{137}$Cs and $^{90}$Sr – in spring, summer and autumn. The milk of cows with higher stress resistance was safer in terms of the content of these components. The data obtained do not contradict the hypothesis that cows with different adaptive abilities may excrete harmful substances with milk at different levels. Therefore, it is advisable to breed dairy herds from animals that are more resistant to stress.

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