

вже на 15 добу після початку лікування. Характерні для дерматомікозів ураження шкіри зникли, в уражених ділянках на шкірі почало відростати волосся. При застосуванні експрес методу діагностики за допомогою ПЕГ 6000 у всіх 5 собак вміст імуноглобулінів був у межах норми.

У тварин другої піддослідної групи за період спостереження було відмічено незначне зменшення наявних уражень, та на 15 добу у 4 тварин був відмічений знижений вміст імуноглобулінів.

Таким чином, при проведенні мікроскопічних досліджень зіскрібків з уражених частин шкіри собак, були виділені культури грибка, які ідентифікували як *Microsporum lanosum*. Водно-спиртовий розчин прополісу при парентеральному (внутрішньомязевому) введенні володіє достатніми імуностимулюючими властивостями. За результатами проведених досліджень водно-спиртовий розчин прополісу володіє добрими лікувальними властивостями та його необхідно застосовувати при лікуванні дерматомікозів у собак.

## LECTINS ISOLATION FROM PROVENDERS AND DETERMINATION OF THEIR ACTIVITY

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**Introduction.** It is known, that some plant anti-nutritional components interfere with the normal absorption of trace elements and nutrients. First of all, these include the widely studied lectins. Numerous studies have shown that lectin proteins negatively affect the absorption, digestion and bioavailability of nutrients, and may contribute to the development of other intestinal diseases, in particular, gastroenteritis.

**Aim.** Methods for the isolation of lectins differ in specificity, speed, price and efficiency. When the salting-out method applied (application of NaCl,  $(\text{NH}_4)_2\text{SO}_4$ , etc.), proteins are precipitated by changing the ionic strength of the solution. This method is simple, does not require special equipment and reagents, and allows to isolate a wide range of proteins by molecular weight. Only part of the precipitated proteins will be lectins, the activity of which is determined in the subsequent test with erythrocytes by the agglutination reaction. Despite its technical simplicity, this method also requires some optimization. The most important parameter is the salt concentration, on which the molecular weight of the extracted proteins directly depends. The aim of this work was to determine the optimal concentration of NaCl to the salt-out maximum amount of lectin proteins.

**Methods.** Isolation of lectins was carried out from a samples of finely ground provenders (30 g) with salt solution (NaCl) (150 ml) in the following concentrations: 0.5%, 0.9%, 1.2%, 1.5%, 1.8%, 2% and 2.5%; at room temperature on a magnetic stirrer for 3-5 hours. The obtained extract was centrifuged for 30 min at 5000 rpm. Sediment and fat droplets were removed from the liquid surface. 100 ml of the extract have been used for further hemagglutination reaction. The hemagglutination activity of the native free lectin complex was determined using a 2-fold dilution procedure using trypsinized piglet erythrocytes. Lectin extract (0.5 ml) was mixed with an erythrocyte suspension (0.5 ml) and incubated at 37 °C for 30 min. The hemagglutination titer was determined as the reciprocal of the highest dilution showing hemagglutination. Total activity was

calculated as the multiplication of titer and volume. To define specific activity (in units – u) the total activity was divided by the protein concentration.

**Results and Conclusions.** Lectin activity was determined in 13 provenders. Several NaCl concentrations have been used for the lectins isolation: 0.5, 0.9, 1.2, 1.5, 1.8, 2, and 2.5 (%). Lupine extract, known for its high lectin activity, was used as a positive control. Also, heat-treated lupine lectin extract served as a negative control. The minimum lectin activity was noted for the extract isolated with a 0.5% NaCl solution (38.4 units of agglutination), the maximum (307.2 u) with a 2% NaCl.

On average, the lectin activity of provenders was 3-8 times lower than the positive control (lupine), which generally indicates their safety and the absence of significant toxic effects. Also, the absence of lectin activity in the thermally treated extract (boiled) of lupine seeds indicates the effectiveness of this method of extraction and neutralization and allows to study specifically lectin activity of provenders extracts.

0.5% NaCl allows to isolate the minimal amount of lectins (on average, 13.21 u). The lowest lectin activity (9.6 u) was found in provenders № 1, 6, 8 and 11. The highest lectin activity was found in provenders № 5 (16 u), 3, 7 and 9 (17.1 u). The provenders with intermediate activity include №12 (11.7 u), №4 and 13 (12.8 u), №2 (13.9 u) and №10 (14.9 u).

Application of 0.8% NaCl allows obtaining lectins with an average of 19.36 u of activity. The minimal activity of lectins was obtained for provenders № 6 (10.7 u), № 1 and 8 (12.8 u) and 4, 12 (14.9 u). The average lectin activity was shown for provenders № 11 and 13 (17.1 u), and 2 (19.2 u). High lectin activity is observed in provenders № 5 and 7 (23.5 u), 3 and 9 (25.6 u), and №10 (34.1 u).

The concentration of 1.2% NaCl was marginally more effective, with 21.01 units of average lectin activity. Most provenders` lectins were found to be weakly active. So, in provenders № 1, 4, 8 and 11, lectins activity was determined at the level of 12.8 u, № 6 - 14.9 u, № 13 - 17.1 u, 2 and 12 - 19.2 u. Only provender №9 (23.5 u) was moderately active, and provender № №5 (25.6 u), №7 (29.9 u), №3 (34.1 u) and 10 (38.4 u) were highly active.

NaCl at a concentration of 1.5% showed an average lectin activity of 22.56 u. A number of provenders have shown low lectin activity: № 11 (9.6 u), № 4 and 6 (14.9 u), 5 and 13 (19.2 u), № 12 (23.5 u). The average activity of lectins was determined in provenders № 1, 2, 9 and 10 (25.6 u) and No. 7 (29.9 u). Only provender № 3 had a high lectin activity at the level of 42.7 u.

Higher efficiency was shown by NaCl at a concentration of 1.8%. Thus, the average lectin activity was 23.71 u. Provenders with minimal lectin activity: № 13 (11.7 u), № 11 (14.9 u), № 8 (19.2 u), № 2, 4 and 12 (21.3 u) and № 6 (23.5 u). The average lectin activity was determined in provenders № 1, 5 and 7 (25.6 u), 9 and 10 (29.9 u). Like 1.5% NaCl concentration, high lectin activity was detected only in provender № 3 (38.4 u).

The average activity of lectins isolated with 2% NaCl was 39.71 u. Lectins with minimal activity were determined in provenders № 13 (21.3 u), 4 and 11 (25.6 u), 5, 6 and 12 (34.1 u). Only provender № 8 was defined with an average lectin activity of 38.4 u. High lectin activity was found in provenders № 7 and 9 (46.9 u), 1, 2 and 10 (51.2 u) and 3 (55.5 u).

The maximum concentration of NaCl at 2.5% allows obtaining an average of 40.04 u of lectin activity. Thus, the provenders with the minimum lectin activity were № 13 (25.6 u), 11 (29.87 u), 4, 5, 8, and 12 (34.13 u), 6 and 9 (38.4 u). Only provender № 1 was identified with an average lectin activity of 42.67 u. High lectin activity was determined in provenders № 2, 3 and 7 (51.2 u) and № 10 (55.47 u).

Application of 2.5% NaCl showed a slightly higher average lectin activity compared to 2% NaCl (40.04 u versus 39.71 u). On the other hand, in some cases, 2.5% NaCl showed a decrease in lectin activity, maximum in the case of positive control - lupine (from 307.2 to 273.07 u), which does not allow us to consider 2.5% NaCl as an optimal reagent for the extraction of lectins. As a result, we could conclude that the application of 2% NaCl allows to isolate the maximum amount of lectins and, thus, is the optimal concentration of NaCl for the lectins isolation.

To determine the activity of lectins in provenders for piglets, the salting-out method was selected. This method is simple, does not require special equipment and reagents, allow to isolate a wide range of proteins by molecular weight. Optimal NaCl concentration was determined at 2% in the subsequent agglutination reaction. We could notice stable results of the optimized technique both in the study of samples with a low activity of lectins (21-25 u) and with relatively high activity (47-55 u). Our results have identified the presence of active lectins in the Belarusian provenders for weaning pigs, their concentration varies significantly (29.85 – 55.5 u) and depends on many factors. We could point, that the activity of lectins in studied provenders was significantly ( $\approx 3$ -8 times) lower than control (307.2 u), which prove the safety of the used provenders. However, this lectin proteins could bind to functional sites in the pigs` intestines and contribute to digestive disorders.

## ЦИТОЛОГИЧЕСКОЕ ИССЛЕДОВАНИЕ ВЫПОТА У КОШЕК БОЛЬНЫХ ИНФЕКЦИОННЫМ ПЕРИТОНИТОМ

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**Актуальность.** Кошачья коронавирусная инфекция может приводить к инфекционному перитониту кошек (ИПК, FIP), который протекает двумя путями:

1) вызывает диссеминированный програнулематозный васкулит — «сухая» форма;

2) вирус FIP размножается в макрофагах, что приводит к осаждению вируснагруженных макрофагов в пределах эндотелия мелких кровеносных сосудов. Далее вирус ИПК накапливаясь в стенках кровеносных сосудов увеличивает их проницаемость, это приводит к выведению белков из кровеносных сосудов и образованию выпотной жидкости — «влажная» форма ИПК.

Вирус инфекционного перитонита кошек поражает домашних и диких животных из семейства кошачьих всех видов. Инфекционный перитонит кошек, наряду с панлейкопенией, вирусной лейкемией и иммунодефицитом кошек является одной из ведущих инфекционных причин смерти у кошек. В последние годы были достигнуты успехи в лабораторной диагностике FIP, но несмотря на значительный