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EVALUATION OF THIAMINE AND RIBOFLAVIN CONTENT IN FEED AND BVD

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The need for the content of vitamins in compound feed are decisive elements that significantly affect the development of the animal's body, their metabolism, reproduction and general condition. Taking into account the nature of vitamins and depending on the ability to dissolve, vitamins are divided into main groups, these are those that dissolve in fats (A, D, etc.), and those that dissolve in water, group B. Adding vitamins to compound feed during fattening animals is difficult to overestimate, because the body, with the exception of vitamins D and B1, does not have the ability to synthesize them, and the lack of vitamins is the cause of various complications and disorders of physiological indicators of animals. Cereal plants are the supplier of almost all representatives of B vitamins (except B12). The content of group B vitamins in cereal crops, which are widely used in feed production, depends on such factors as genotype, methods of processing before the production of compound feed, storage conditions, and others.

The quantitative determination of vitamins and the use of modern methods for the formation of a balance of vitamins in compound feed and BVD is an urgent task of manufacturers engaged in the production of compound feed for animals and poultry.

Key words: *thiamine, riboflavin combined feed, BVD, methods, analysis, substance.*

PROBLEM

For the quantitative determination of vitamins of group B, it is necessary to break down the complexes and isolate the studied vitamin in a free form available for physicochemical analysis. The release of thiamine from the bound state can be carried out with the help of acid hydrolysis and under the influence of phosphotase and proteolytic enzymes.

ANALYSIS OF THE LATEST RESEARCH

Vitamins of group B are necessary for the formation of a healthy animal body and ensuring reproductive functions. Vitamins of this group should be supplied to animals together with the feed they will receive during fattening. Group B vitamins create conditions for obtaining the necessary energy, preventing the occurrence of pathologies, skin diseases [1,3,4].

Analysis of vitamin content is carried out to evaluate compound feed recipes and confirm the correct regime of animal fattening. Vitamins of the specified group that entered the body with food remain in the required amount, and the excess is excreted in the urine. Due to the fact that these vitamins are water-soluble, a small amount of them remains in the body, so it is necessary to ensure the enrichment of feed with such substances.

A possible lack of vitamin B is a consequence of:

- lack of vitamin B in feed;
- disadvantages of fattening regimes
- the animal receiving drugs that prevent the accumulation of vitamin B;
- lack of vitamins of another group.

Vitamin B2 (riboflavin) is a coenzyme that contributes to the accumulation of necessary energy, the metabolism of lipid composition and almost all B vitamins, and promotes blood circulation. The lack of riboflavin is the reason for the occurrence of heart rhythm disorders, a decrease in immunity, the development of anemia and weakness of animals [2,3,4]. Ensuring the functioning of the body and digestive tract of animals,

they should be provided with high-quality and balanced feed, based on this, the main requirements for feed arise, which should provide:
- compound feed must meet the needs of animals and poultry, have a sufficient amount of raw cellulose;
- feed must meet the requirements for the content of vitamins, proteins, and minerals;
- feed must meet the energy needs of animals;
- feeding should provide the body with nitrogen and easily digestible carbohydrates.

RESEARCH RESULTS

An express fluorometric method of analysis based on the oxidation of thiamine to thiochrome and measurement of the fluorescence intensity of thiochrome is used for the quantitative determination of vitamin B in compound feed and BVD. The method of determination is as follows. The average sample of the product under study is ground on an electric laboratory mill, a suspension of 5 g is taken, weighed on an analytical balance, and transferred to a 100 ml volumetric flask. Add 50 ml of 1 N sulfuric acid and place in a boiling water bath for 40 minutes for hydrolysis, stirring periodically. After that, the flask is cooled and the solution in it is brought up to the mark with distilled water. The centrifuge is separated from the sediment and filtered through a pleated filter. Take 20 ml of the filtrate, pass it through an adsorption column with cationite for 10 minutes to remove fluorescent impurities. After that, the column is washed with distilled water until the universal indicator reacts neutrally. Vitamin B1 is eluted with a boiling solution of 25% potassium chloride in 0.1 N hydrochloric acid to a volume of 30 ml in portions of 5-6 ml for 10 minutes with stirring. Oxidation of vitamin B1 into thiochrome is carried out in five 100 ml round-bottomed flasks. The following amount of reagents, 1st and 2nd, 1 ml of a standard solution of vitamin B1 with a titer of 1 • 10 -5 g/ml, 3 ml of distilled water, 1 ml of a 0.1 N solution of potassium chloride in hydrochloric acid are added to each flask. , 1.5 ml of the oxidizing mixture, 1.5 ml of 15% NaOH. 3rd and 4th, 1 ml of the test solution, 4 ml of distilled water, 1.5 ml of the oxidizing mixture. 5-a, 1.5 ml of 15% NaOH. The oxidizing mixture is prepared 2-3 hours before the start of the analysis. Mix 5 ml of an aqueous solution of ferric potassium and 25 ml of 15% sodium hydroxide. The contents of the flasks are mixed well for 1 min. Extraction of vitamin B1 is carried out with N-butyl alcohol, pouring 12 ml into all flasks. The flasks are thoroughly shaken for 3 min and the layers are allowed to separate in a dark place. The upper alcohol layer is carefully poured into 100 ml conical flasks, which are previously filled with 0.5 g of anhydrous sodium sulfate. The alcohol layer is dried for 10-15 minutes and poured into the cuvettes of the fluorometer. The calculation of the content of vitamin B1 is carried out according to the formula:

$$X = \frac{(A-B)T\ Vn\ Vp\ 10^6}{(A_1-B_1)Vr\ Vok\ P} \quad (1)$$

where:
X - content of vitamin B1 in g per 1 ton of BVD;
A - the average of the readings of the fluorometer for two parallel tested solutions;
B - reading of the fluorimeter for the control sample to the tested solution;
A_1 - reading of the fluorometer for the standard solution;
B_1 - reading of the fluorimeter for the control sample to the standard solution;
Vg - amount of hydrolyzate taken for adsorption, ml;
Vp - dilution of eluate, ml;
Vok- taken for oxidation, ml;
Vn- dilution of BVD overhang, ml;
R - overhang of BVD, g;
T - titer of the standard solution, g/ml;
10^6 - recalculation for 1 ton BVD.

Vitamin B2 (riboflavin). An express method based on the intrinsic fluorescence of riboflavin and its derivatives has been developed to determine the content of riboflavin in compound feed. To isolate riboflavin from

compound feed, acid hydrolysis of 1 N sulfuric acid is used followed by treatment with a 20% solution of trichloroacetic acid to split the bound forms. This allows you to exclude enzymatic hydrolysis, which reduces the analysis time to 1 hour. The method of rapid determination of riboflavin content is as follows. The average sample of compound feed is ground in an electric laboratory mill. A 5 g sample is transferred to a 100 ml volumetric flask, 50 ml of 1 N sulfuric acid is added and placed in a boiling water bath for 20 minutes. Bring the volume up to the mark with hot distilled water and filter while hot. The flask is rinsed with 3.5 cm^3 of cold distilled water and poured onto the filter (adjusted to a volume of 100 ml), then the filtrate is mixed. To determine vitamin B2, take 30 cm^3 of the filtrate, add an equal volume of 20% trichloroacetic acid solution and put it in a boiling water bath for 10 minutes. After cooling, adjust the pH of the resulting solution according to the universal indicator to 4-4-4.5, with a 40% caustic sodium solution of about 5 cm^3, filter through a pleated filter, take 10 cm^3 of the resulting filtrate into fluorometer cuvettes and fluorometer. The fluorescence of the obtained solution is compared with the fluorescence of the working standard solution of riboflavin, the titer of which is equal to 4 • 10-7 g/cm^3. Preparation of a standard solution of vitamin B2 is carried out as follows. A weight of 0.04 g of riboflavin is dissolved in hot distilled water in a volumetric flask with a capacity of 1,000 cm3 while heating in a water bath. After cooling, the solution is brought up to the mark with water. The solution is stored in the dark in the cold for a little over a month. Before the analysis, a working standard solution is prepared: 1 cm^3 of the main standard solution is taken into a 100 cm^3 volumetric flask and brought up to the mark with water. The titer of the obtained standard working solution is 4•10-7 g/cm^3. After measuring the fluorescence, 0.1 g of $NaHCO_3$ and 0.1 g of $Na_2S_20_4$ are added to each cuvette to quench riboflavin fluorescence, and the fluorescence intensity is measured again. In the standard solution, the fluorescence is quenched to zero. In the studied solutions, there remains a small fluorescence caused by extraneous fluorescent substances. When calculating the amount of riboflavin, this fluorescence is subtracted. Fluorescence quenching is repeated once more to ensure that the riboflavin is completely destroyed.

The calculation of the content of vitamin B2 is carried out according to the following formula:

$$X = \frac{(A-B)TVn\ Vp\ 100\ \ 1000}{(A_1-B_1)Vr\ q} \quad (2)$$

Where

X- is the content of vitamin B2 in mg, %;

A - reading of the fluorometer for the tested solution;

B - reading of the fluorometer for the investigated solution after fluorescence quenching;

A1 - reading of the fluorometer for the standard solution;

B1- fluorimeter reading for the standard solution after fluorescence quenching

T - titer of the standard solution;

Vn - dilution of overhang, cm^3;

Vp - dilution of hydrolyzate, cm^3;

Vr - amount of hydrolyzate for additional hydrolysis with trichloroacetic acid, cm^3;

q - overhang of riboflavin, g;

100 - conversion into percentages;

1000 - conversion to mg.

CONCLUSIONS

The application of the presented determination mechanism also makes it possible to determine the content of vitamin B1. Determination of the content of vitamins B1 and B2 can be performed within 2 hours, which significantly reduces the time spent on conducting research.

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ОЦІНКА ВМІСТУ ТІАМІНУ ТА РИБОФЛАВІНУ В КОМБІКОРМАХ ТА БВД

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Необхідність вмісту вітамінів в комбікормах є вирішальними елементами які суттєво впливають на розвиток організму тварин, їх метаболізм, репродукцію та загальний стан. Враховуючи природу вітамінів та залежно від здатності розчинятися, вітаміни поділяють на основні групи, це такі , що розчиняються у жирах (А, D,та ін.), та такі, що розчиняються у воді, група В. Додавання вітамінів у комбікорми під час відгодівлі тварин важко переоцінити, тому, що організм, за виключенням вітамінів D та В1, не має здатності здійснювати їх синтез, а нестача вітамінів є причиною різних ускладнень та розладу фізіологічних показників тварин. Постачальником майже всіх представників вітамінів з групи В (за виключенням В12), є злакові рослини. Вміст вітамінів групи В у злакових культурах, які масово використовуються у виробництві кормів, залежить від таких чинників як генотип, способів обробітку перед виготовленням комбікормів, режимів зберігання, та інших.

Кількісне визначення вітамінів та використання сучасних методів для утворення балансу вітамінів у комбікормах та БВД є актуальною задачею виробників зайнятих в області виготовлення комбікормів для тварин та птиці.

Ключові слова: *тіамін, рибофлавін комбікорм, БВД, методи, аналіз, речовина.*