Evaluation of the effectiveness of highly dispersed metal powders (Ca, Cu, Zn, Fe) used to increase digestibility and bioavailability of feed substrates

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Abstract. The paper presents the results of research on feed additives based on wheat bran including highly dispersed metal powders (Ca, Cu, Zn and Fe) in various combinations subjected to extrusion processing. Physical properties (porosity) of the experimental feed substrates were assessed by the method of determining the porosity of acetone in accordance with GOST 6217. To determine feed digestibility and bioavailability of elements in vitro, an “artificial rumen” was used. The elemental composition of feed samples was studied by atomic emission spectrometry with an inductively coupled plasma (AES-ICP). Finely dispersed powder of Ca (up to 10 μm), Cu, Zn, Fe (9-10 μm) was studied. Digestibility of dry matter of feed substrates was dependent on their porosity. Extrusion of bran products increased dry matter digestibility by 6.8%, and addition of fine powder increased it by 14% (P≤0.05) in vitro conditions. An increase in bioavailability of minerals after extrusion of Cu, Zn and Fe by 19.3, 6.3 and 23.1% (P≤0.05), and after addition of highly dispersed metals by 2.98 (P≤0.05), 21.6 and 7.3% (P≤0.05) was identified.

1. Introduction

The problem of micronutrient deficiencies in feed causes serious diseases of farm animals, decreases intensity of their growth and reproductive qualities [1, 2], and their content in final animal products intended for human consumption. Development and introduction of new technologies for enriching products by transforming macro- and microelements into bio-accessible mineral systems for agricultural animals and humans is a promising direction [3].

In the last decade, new forms of biologically active substances required for development of animals and their productivity have been identified [4]. Highly dispersed metals can be used for this purpose [5]. The use of the latter will increase their bioavailability, ensure low toxicity and prolonged action [6, 7].

At the same time, the effectiveness of preparations containing particles of highly dispersed metals can be increased by exposing them to various physical methods, including extrusion. According to modern concepts, extrusion of concentrated feeds increases nutrient absorption, improves sensory characteristics and extends the shelf life of feeds [8].

However, prospects for the extrusion method in the production of feed including mineral substances are vague.

Studies of the effectiveness of feed compositions based on bran products subjected to extrusion processing in combination with highly dispersed metals are of great interest.
2. Materials and methods

2.1 Research object

Bulls of the Kazakh white-headed breed aged 13 months, rumen liquid.

Experimental studies were performed in accordance with the instructions and recommendations of the Russian Regulations, 1987 (Order No.755 on 08/12/1977 by the USSR Ministry of Health) and "The National Academy Press Washington, DC 1996). Efforts were made to minimize animal suffering and reduce the number of samples used.

2.2 The scheme of the experiment

The study was conducted under the conditions of the Center for Collective Use of the Federal Research Center of Biological Systems and Agrotechnologies of the Russian Academy of Sciences.

The rumen fluid was taken from the bulls through chronic rumen fistula. Studies were performed by the method of nylon pouches: in vitro - using the "artificial rumen KPL 01", 48-hour exposure.

The following experimental systems were used as research objects: native wheat bran (OK1), extruded wheat bran (OK2), extruded bran mixture 80% + VDP Ca 20% (OK3), extruded bran mixture 79.9% + CaP 20% + VDP Cu, Fe, Zn 0.1% (OK4), extruded bran mixture 99.9% + VDP: Cu, Fe, Zn 0.1% (OK5).

For the research purpose, we used wheat bran with crude fiber content - 8-10%, crude protein - 13-15%, with a particle size of up to 0.5 cm. Highly dispersed powders (VDP): Ca - particles less than 10 microns, Cu, Zn, Fe produced by Alfa Aesar GmbH & Co KG, a particle size - 9-10 microns, purity - 99.7% (EEC No. 231-096-4) (Germany).

The dosage of the VDP added into the feed substrates was calculated on the basis of 1 kg of extrudate: Ca - 200 g, Zn - 0.1 g, Fe - 2 g, Cu - 0.1 g.

During the study, mixtures of bran and Ca VDP were prepared at the following ratio: wheat bran - 80% and Ca VDP - 20%; wheat bran - 79.9% and CaP - 20%, VDP of metals 0.1% (Zn - 0.1 g, Fe - 2 g, Cu - 0.1 g) which were subjected to extrusion. To obtain an extrudate, a universal single-screw press extruder PESH-30/1 (Russia) with a capacity of 45 kg/h. was used. Pre-moistened (moisture content of 30%) mixture samples were extruded at a power of 7.7 kW, pressure of 10 MPa and temperature not exceeding 120 °C. The volume of water for moistening the samples was determined by formula (1).

\[
V = m \times (W_2 - W_1)/(100 - W_2)
\]  

\(V\) – the volume of water required to moisten the samples, ml;  
\(m\) – sample mass, g;  
\(W_1\) and \(W_2\) – initial and final mass fractions of moisture, %.

Estimation of orosity of the raw material assumed the use of feed in an air-dry state placed in a measuring cylinder \((V = 100 \text{ ml}, d = 25 \text{ mm})\) with a feed density of up to 300 ± 10 g/l. The cylinder was weighed with an accuracy of 0.01 g and filled with acetone to a constant level of acetone over the sample layer. After 30 minutes of exposure, the excess acetone was merged, and the cylinder was weighed. Acetone porosity (%) was calculated as adifference using formula (2).

\[
X = \frac{(G'_{\text{la}}-G'_{\text{lb}}) \times 100}{p \times V} = \frac{G''_{\text{la}}-G''_{\text{lb}}}{p}
\]  

\(G'_{\text{lb}}\) – weight of the cylinder with the sample before impregnation, g;  
\(G''_{\text{la}}\) – weight of the cylinder with the sample impregnated with acetone, g;  
\(p\) – acetone density at a test temperature, g/cm³;  
\(V = 100 \text{ cm}^3\) – sample volume.

Dry matter digestibility was determined by the in vitro method, using an “artificial rumen KPL 01”, a thermostat of electric dry-air TC-1/80 SPU (Smolensk SKTB SPU OJSC, RF); a laboratory electronic scales VM 153 (OKB Vesta LLC, RF); a gastric probe (Russia).

A portion of the feed additives was placed in bags made of polyamide fabric. The rack with fixed bags dropped into a mixture of rumen juice and a buffer solution and placed in a thermostat for 48 hours at a temperature of +39 °C. Then the bags with samples were placed in the diluted pepsin and the device was placed in the thermostat for 24 hours for secondary digestion. At the end of digestion with pepsin, the samples were taken out of the bath, thoroughly washed with tap water and dried at a temperature of +60 °C to a constant weight. Then the degree of feed digestibility was calculated using formula (3).
\[ K = \left( \frac{A - B}{C} \right) \times 100 \% \]  

(3)

\( K \) – dry matter digestibility coefficient (%);

\( A \) – the initial mass of the feed sample together with the bag (g);

\( B \) – the mass of the feed sample together with the bag after digestion (g);

\( C \) – the initial mass of the sample feed without the mass of the bag (g).

Bioavailability assessment was carried out according to the in vitro results as a percentage of the difference in the content of the evaluated elements, before and after exposure.

The elemental composition of the feed was determined by atomic emission spectrometry with an inductively coupled plasma (AES-ICP) in the laboratory of the autonomous non-profit organization “Center for Biotic Medicine”, Moscow.

2.3 Statistical processing

Data are expressed as mean values ± standard error of the mean. Statistical analysis was performed using Statistica 10.0 (StatSoft Inc., USA) and Microsoft Excel (Microsoft, USA). Significance of the group differences was estimated using Student’s t-test with \( p \leq 0.05 \) considered as significant.

3. Results

The analysis showed that porosity of extruded wheat bran increased by 8.8% (\( p \leq 0.05 \)).

It was established that mineral additives in the form of the VDP did not have a pronounced effect on the estimated index (Fig. 1).

![Figure 1](image.png)

* - \( p \leq 0.05 \) in relation to OC1

The results of in vitro evaluation of the VDP effect on digestibility are presented in Figure 2.
Figure 2. In vitro digestibility of dry matter of the tested food, % * - P≤0.05 in relation to OC1

In vitro evaluation of feed digestibility revealed a significant change due to the extrusion. The share of digestibility of native bran was 64.2%, digestibility of extruded bran increased by 6.8%. The addition of VDP (Ca, Cu, Zn and Fe) into the extrudate increased the digestibility of dry matter by 14% (P≤0.05).

The comparative analysis of the results between groups of extruded feeds showed that addition of CaD, Cu, Zn and Fe VDP increased digestibility of the substrate by 1.2% more than Ca, and by 5.8% more than Cu, Zn and Fe.

Calculation of bioavailability of metals from experimental preparations showed an increase in this parameter for Cu by 19.3%, Zn - by 6.3%, and Fe - by 23.1% (P≤0.05) (Fig. 3).

Figure 3. Bioavailability of in vitro microelements from the tested food, % * - P≤0.05 to OC1
Ca, Cu, Zn, and Fe in the extruded mixture increased bioavailability. Thus, the maximum increase for Cu was 2.98% (P≤0.05), for Zn - 21.6% and for Fe - 7.3% (P≤0.05).

4. Discussion
In order to form biologically complete diets, scientists seek to develop highly effective feed additives. Highly dispersed mineral powders can be used as an alternative to existing drugs in animal feeding [10-12].

When planning the experiment, we relied on the results of previous studies which justified the addition of highly dispersed metal powders and their dosages into the feed for farm animals and birds and described positive effects of these substances [13-15].

Digestibility of dry matter through the extrusion process increased by 6.8%; addition of VDP increased digestibility by 14% (P≤0.05). The data on digestibility are confirmed by the results obtained by a number of authors [16]. This process may be due to the structural transformation in the experimental feed due to the extrusion [17]. Dietary fibers and VDP undergo chemical modification changing properties of the feed [18]. High digestibility of nutrients may be due to physical transformations under the action of extrusion and the biological effect of highly dispersed metal particles [19]. This is consistent with previous studies which showed that feeding with highly dispersed particles improves digestive processes, increases immunity and productivity of farm animals [20].

In addition, VDPs have a number of advantages, including high bioavailability, stability of interaction with other components, lower toxic effects, since absorption of particles occurs as a result of enteral digestion [21]. This is confirmed by our data on the assessment of bioavailability of chemical elements. We found a significant increase in this indicator in the extruded sample with a highly dispersed complex for all the elements (Fig. 3). The fact can be explained taking into account that an increase in bioavailability of mineral elements from the composition of experimental feed additives is due to the increased activity of the microflora of the digestive tract and partial destruction of raw fibers surrounded by highly dispersed metal particles [22-27]. In addition, the use of highly dispersed systems makes it possible to facilitate the penetration of mineral substances or biologically active compounds through biological barriers, as well as to avoid metabolic modifications that could cause low absorption [28-31].

The composition of the digested food matrix, synergism and antagonism of various components, as well as various physicochemical properties of materials [32] are factors influencing bioavailability of feed. However, these processes should be studied.

5. Conclusion
Thus, the wheat bran extrusion in combination with Ca, Cu, Zn, Fe VDP changes the structural and chemical composition of the experimental complex, increasing digestibility of nutrients and bioavailability of minerals. It was also found that an increase in digestibility of dry matter occurs by increasing the porosity of the feed substrate.

6. Acknowledgments
The study was performed in the framework of the project #0761-2019-0005.

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