Supramolecular complexes of drugs for parasitic sheep invasions

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Abstract. The complex of multicellular parasites of sheep in Gorny Altai is mainly represented by intestinal and pulmonary strongylates, moniesia, dicrocelia and parasitic insects, which involves the use of complex parasiticidal agents of a wide spectrum of activity in the system to minimize the volume and frequency of use of drugs. Ivermectin (IVM) has a nematodocidal and entomocidal effect, fenbendazole (FBD) is an effective nematodocide and cestodocide, triclabendazole (TBD) is a recognized trematodocide. To obtain more effective drugs based on these substances, their mechanochemical modification using polyvinylpyrrolidone (PVP) is proposed. The aim of the work is to show the promise of using mechanochemical technology to improve the physicochemical and biological properties of drug substances and to evaluate the effectiveness of compositions of supramolecular complexes of polyvinylpyrrolidone with IVM, FBD and TBD in parasitic sheep infestations. Experiments on the study of the parasitocidal activity of compositions for intestinal helminthiases were carried out on a flock of spontaneously invasive sheep in the Shebalinsky district of the Altai Republic. According to the principle of analogues, 6 experimental (10 animals each) and 2 control (20 animals each) groups were formed. The preparations were given to the sheep individually in the form of an aqueous suspension orally. The study demonstrated the high efficiency of solid dispersion (SD) of the composition FBD / IVM / PVP at a dose of FBD - 3.0, IVM – 0.2 mg / kg b / w when administered orally against gastrointestinal strongylates and moniesia (95.8 and 100%) and a bloodsucker in sheep (92.5%). SD of the composition TBD / IVM / PVP at a dose of TBD - 3.0, IVM – 0.2 showed high efficacy against gastrointestinal strongylates and dicrocelia (96.8 and 100%) and was not effective against moniesia and sheep blood suckers. The initial substances of FBD and TBD showed significantly less activity during parasitic infestations of sheep. The use of the studied SD suggests the possibility of a significant (three-fold) reduction in the dosage of FBD and TBD without loss of parasiticidal activity.

1. Introduction

The sheep zoo in the south of Western Siberia is characterized by significant diversity and is represented by almost all major classes causative agents by nematodes, trematodes, cestodes, insects, arachnids and Pentastomes, taking into account rare nosoforms, this is more than 30 diseases. So, the parasitocomplex sheep in the Altai Mountains are mainly represented by gastrointestinal and pulmonary strongylates (family Ostertagia, Trichostrongylus, Nematodirus, Haemonchus, Protostrongylus, Dictiocaulus, etc.), moniesia (Moniezia benedeni Blan., M. expansa Blan.), dicrocelia (Dicrocelium lanceatum St. et Has.),
sheep gadfly (*Oestrus ovis L.*) and sheep bloodsucker (*Melophagus ovinus L.*) and to a lesser extent other parasites [1-3]. Prevailing epizootic the situation involves the involvement in the system of measures of complex parasitocidal broad-spectrum agents.

Studies have shown that parasitocenosis of farm animals in various landscape-geographical conditions includes a specific range of pathogens diseases to be controlled. In many cases, there are representatives several main classes of multicellular parasites against which at present time there is no integrated effective parasitocide. Real epizootic situation involves the involvement in the system of events of 2-3 new broad-spectrum antiparasitic agents suitable for use and affordable enough for the economically weak majority agricultural producers. In addition, the minimization factor interference with the parasitic system, reducing the frequency of manipulations with animals suggest the use, as therapeutic agents, of complex preparations, with a wide spectrum of action [2, 4-6].

In recent years, new antiparasitic drugs have been developed and proposed for use. The drugs have a wide spectrum of action against many endo- and ectoparasites of animals and birds. These drugs include anthelmintics containing as an active substance (AI) macrocyclic lactone, which are highly effective therapeutic agents against nematodes and ectoparasites animals and benzimidazoles with activity against a wide range of helminths [6-9]. The combination of AI of these drugs in a complex tool allows control an extensive range of parasites.

In various natural provinces of the Altai Mountains, ruminants formed corresponding zoo parasitic complexes represented by dominant classes parasites: nematodes (Nem) + parasitic insects (Ent); Nem + Ent + Cestodes (Ces) and Nem + Ent + Trematodes (Tr). To control ones, it is necessary AI in all combinations of nematodocides and entomocides, with the addition of cestodocides and trematodocides. Ivermectin (IVM) fully meets these requirements with possessing of nematodocidal and entomocidal action, fenbendazole (FBD) is effective nematodocide and cestodocide, triclabendazole (TBD) is recognized trematodocide. Due to their poor solubility, their AI used in drugs is often not provide high efficiency and are used in excessive dosages.

The increase in solubility when creating parasitocides is provided by mechanochemical modification of these AI with water-soluble polymers, in particular, with polyvinylpyrrolidone (PVP). With this treatment, solid dispersions (SD) are obtained in the form of thin, easily flowing powders forming supramolecular complexes in water with increased solubility, bioavailability and effectiveness, which in turn gives the possibility of a multiple reduction in the dosage AI of drugs.

Pharmacological improvement properties of drugs is explained by targeted transport to a given area, organs or cells, both the host organism and the parasite [10-12].

The aim of this work is to show the prospects of use mechanochemical technology to improve physico-chemical and biological properties of IVM, FBD and TBD and evaluate the effectiveness of their supramolecular complexes with PVP in parasitic infestations of sheep.

2. Materials and methods

2.1. Chemical and technological specification

For actual researching were used:

- the substance of ivermectin (IVM) series 0315110205 with a content of AI (22,23-dihydroavermectin B1a + 22,23-dihydroavermectin B1b) equal to 97.5%. Produced by Shandong Qilu King-Phar Pharmaceutical Co. Ltd. (PRC). Its solubility in water is 4.0 mg / L.
- the substance of fenbendazole (FBD) with a content of AI /[6-(phenylthio)-1H-benzimidazole-2-il] carboxylic acid methyl ester/ equal to 98.0%. Produced by Renzin Chemicals Ltd. (PRC). Its solubility in water is 0.1 mg / L.
- substance of triclabendazole (TBD) of the SZBC22XV series with a content of AI /5-chloro-6-(2,3-dichlorophenoxy) -2- (methylthio) -1H-benzimidazole / equal to 99.7%. Produced by Sigma Oldrich. Its solubility in water is 1.0 mg / L.
• polyvinylpyrrolidone (PVP) – by brand FSP 42-0345-4368-03 with a molecular weight of Mw \(~\) 12 kDa.

Mechanochemical processing of substances FBD and TBD with PVP at a weight ratio components 1/9, was carried out in a metal drum mounted on a roll mill LE-101 (Hungary) with a process module of 1:16 according to the known technology [13]. Joint mechanochemical processing of substances FBD and IVM with PVP, as well as TBD and IVM with PVP at a component ratio of 1/1/9 was carried out by the same method. The SD obtained by machining were free-flowing beige powder, soluble in water with the formation of supramolecular complexes [14].

Samples of antiparasitic compositions were prepared in the form of dry suspension concentrates (DSC) from SD composition FBD / PVP (1/9), TBD / PVP (1/9), FBD / IVM / PVP (1/1/9), TBD / IVM / PVP (1/1/9) of the filler and suspension stabilizer (sodium salt arboxymethyl cellulose / blanose /). The obtained DSC samples are fine beige powders that are stored in plastic bags. After adding water to them, a stable aqueous suspension is formed, ready for use.

2.2. The study of the parasitocidal activity of drugs
The study was conducted on the principle of “control test”, was randomized and placebo-controlled. Conducted in accordance with the Guidelines for experimental (preclinical) study of new pharmacological substances [15] and regulations adopted by the European Convention for the Protection of Vertebrate Animals Used for experimental and other scientific purposes [16]. The sheep used in the experiments were spontaneously (naturally) infected with intestinal strongilates, moniesia, dicrocelia and bloodsuckers.

2.2.1. Helminthiasis Sheep. In the experiment we used one hundred 6-8-month-old sheep of the Gorno-Altai breed weighing 25-30 kg, spontaneously infected with gastrointestinal strongylates and Moniezia spp. of private economy "Individual Entrepreneur V. Yu. Chichinova" at Shebalinsky district of the Altai Republic. During the experiment, the sheep did not graze in the pasture, but were kept indoors and fed on norms and diets of livestock feeding [17]. During the experiment, water to animals provided plenty.

3 days before the experiment, feces were studied by flotation method according to Kotelnikov-Khrenova, a method of successive washes with counting intestinal eggs helminths and dicrocelia in the VIGIS chamber [18].

Then the sheep were randomly assigned to experimental groups with a similar the degree of infection to further determine the proportion of infected animals (%), medium the arithmetic and geometric number of eggs per gram of feces (e./ g.f.) [18, 19].

2.2.2. Sheep melophagosis. In the experiment, the same animals were used as in the study of the effectiveness of helminthiasis (2.2.1.). 3-4 days before the experiment, the animals were examined on infection with adult forms of sheep’s bloodsucker (Melophagus ovinus L.). All the fleece of sheep was examined, dead individuals of bloodsuckers were not taken into account. According to the results of tests determined the proportion of infected animals (%), arithmetic mean and geometric mean of the number of bloodsuckers on an animal.

2.2.3. Experimental groups. Sheep naturally infected with intestinal helminths and a bloodsucker were random way divided into 2 series of experiments. In each series, 3 experimental groups of 10 goals in each and 1 control group of 20 animals. In the first series experiments were tested DSC based on SD of FBD/IVM, in the second series - TBD and IVM in underestimated dosages of Al of benzimidazoles. Anti-parasitic compositions based on DSC preparations were administered orally to animals experimental groups in the form of a 5% aqueous suspension at a dosage of FBD and TBD at 3 mg / kg body weight (mg / kg b.w.) of the AI, IVM - 0.2 mg / kg.
The sheep of the experimental groups were also injected with the initial preparations of FBD and TBD without their mechanochemical treatment in a dosage of 3 mg/kg b.w. Sheep control groups was administered a 0.5% suspension of blanose at a dose of 1.0 mL per kg of body weight (mL/kg b.w.).

2.2.4. Animal treatment. The sheep of the experimental groups were weighed and tagged before setting up the experiments (numbered). Feces for helminth infection were taken directly from the rectum of animals. The effectivity of samples of compositions of parasiticides against gastrointestinal strongylates, moniesia and sheep wild boar was determined by the results of coproscopic examinations of animals with the calculation of the proportion of infected animals (%), arithmetic average and average geometric values of the number of helminth eggs in the samples before, after 15 (strongylata and moniesia) and 30 days (dicrocelia) after deworming of animals of experimental and control groups.

The effectivity of the compositions of the preparations against sheep bloodsuckers was determined on the 15th day after drug administration by examining the fleece of sheep from the experimental and control groups animals with the calculation of geometric mean values of the number of live parasites.

Studies were conducted in accordance with recommendations for evaluating the efficacy anthelmintics in ruminants (cattle, sheep, goats) World Association for the Development of Veterinary Parasitology [19].

The clinical parameters of sheep, such as temperature, frequency, were studied heart rate, respiratory rate, scar movement and behavior. Animals were examined before and after the first, third and fifth day of taking the drug. Research was done every day in the morning before feeding according to the methodology of veterinary-clinical laboratory diagnostics [20].

2.2.5. Statistical Data Analysis. During coprological examinations, indicators of infection were displayed:

- EI,% - the extensity of the invasion, the proportion of infected animals;
- ANE - the arithmetic mean and geometric mean of the number of eggs in 1 gram of feces (e/g. f.) per one examined animal; arithmetic mean and geometric mean of the number of bloodsuckers on the animal.

To assess the parasitocidal activity of the drugs, the following indicators were calculated:

- EE, % - the extensity of efficacy, the proportion of released animals from parasites by relation to control (not processed);
- IE, % - intensity of efficacy, decrease in the average number of eggs in relation to control (not processed) according to the formulas proposed by A. Nepoklonov and G. Talanov [21].

Also, to compare the parasitocidal efficacy of the compositions, a statistical analysis of data on the geometric mean of the number of helminth eggs or parasites (Ef), a parametric t-test was used to compare the differences between experimental and control groups of animals at a significance level of P≤ 0.05. Calculations carried out using SAS / Stat software (SAS version 9, System for Windows).

3. Results of researching

The solubility analysis of the obtained compositions of FBD and TBD with PVP confirmed an increase of solubility 14-29 times, which was expected to affect the anthelmintic the effectivity of the studied compositions (see table 1 and table 2).

| No | Sample, its composition and conditions for obtaining | Solubility absolute, mg / L | Increase, multiplicity |
|----|--------------------------------------------------|----------------------------|-----------------------|

**Table 1.** Water solubility of samples based on FBD.
Analysis of the data in table 1 showed that even the physical mixture (without machining) starting components (FBD and PVP) has a higher solubility value, which can be explained by the solubilizing properties of the hydrophilic polymer, which is a PVP. The machining of a mixture of FBD with PVP leads to a significant increase solubility. However, an increase in machining time after 8 hours does not lead to a significant increase in the time of mechanical modification of the substance FBD by polymer. Therefore, the optimal machining time to obtain the target product - SD composition FBD / PVP (1/9) - selected time 8 hours.

Table 2. Water solubility of samples based on TBD.

| No | Sample, its composition and conditions for obtaining | Solubility absolute, mg / L | Increase, multiplicity |
|----|---------------------------------------------------|-----------------------------|-----------------------|
| 1  | TBD - literature data                             | 1.0                         | -                     |
| 2  | TBD-initial sample without mechanical treatment (m/t)^a | 2.0                         | -                     |
| 3  | Physical mixture of TBD|PVP (1/9) without m / t      | 2.3                    | 1                     |
| 4  | SD of composition TBD/PVP (1/9) after 2 hour m/t  | 11.6                       | 4                     |
| 5  | SD of composition TBD/PVP (1/9) after 4 hour m/t  | 18.1                       | 9                     |
| 6  | SD of composition TBD/PVP (1/9) after 8 hour m/t  | 34.5                       | 17                    |
| 7  | SD of composition TBD/PVP (1/9) after 16 hour m/t | 57.6                       | 29                    |
| 8  | SD of composition TBD/PVP (1/9) after 20 hour m/t | 57.1                       | 29                    |

^a Machining.

Analysis of the data in table 2 allows us to talk about similar trends with increase in table solubility with increasing machining time. However, the optimal time to obtain a soluble SD composition of TBD / PVP (1/9) is not less than 16 hours machining, which is probably associated with the peculiarities of physic & chemical properties of TBD.

For SD compositions of FWD / IVM / PVP and TBD / IVM / PVP such a significant increase of solubility of substances was not observed (only 3-5 times), apparently due to a lack of PVP.

In clinical trials, no harmful effects of drugs and substances on animal health were not found. All parameters matched physiological norms characteristic of this animal species.

In a series of experiments with FBD and IVM, when examining 20 sheep of the control group, that, with gastrointestinal strongylates, animals are infected by 85.0% with an average (arithmetic) value of the number of eggs 650.5 ± 82.4 ins. and the geometric mean 2.9 e. / g.f. In a series of experiments with TBD and IVM, the sheep of the control group are infected gastrointestinal strongylates by 80.0% with an average number of eggs of 484.2 ± 77.2 ins. And the geometric mean 2.69 e. / g.f. (see table 3, No. 1 and No. 5).

In the experimental group when testing the SCS composition FBD / IVM / PVP with strong gastrointestinal tract (see table 3, No. 2) all performance indicators were 100%, while FBD/PVP (No. 3) showed a rather high activity, the indicator of EE - the decrease in the proportion of infected animals was 88.3%, an indicator IE - a decrease in the number of eggs in samples was 99.7%, a decrease in
geometric mean the number of eggs (Ef) 92.1% at P < 0.001. FBD substance (No. 4) in the same dosage (3.0 mg / kg b.w.) was not active enough, the EE was 41.2%, IE - 67.7%, Ef - 17.3% at P > 0.05.

In the experimental group, when testing an DSC of the composition of TBD / IVM / PVP with strong gastrointestinal tract (see table 3, No. 6) all performance indicators also amounted to 100%. DSC of composition TBD / PVP (No. 7) in the experiment showed low activity, the EE was 25.0%, IE - 27.5%, Ef 0%. Substance of TBD (No. 8) in a dosage of 3.0 mg / kg b.w. in case of gastrointestinal strongylatoses infections was ineffective, EE - 0%, IE - 15.3%, Ef - 0%.

Table 3. Efficiency of DSC of parasitocides in sheep gastrointestinal strongylatoses.

| №   | Group of animals | Preparation Dose, mg of AI /kg b.w. | Numb er of sheep in group | EI , % | ANE<sup>a</sup> e / g.f. | EE, % | IE, % | Effica
|     |                 |                                    |                           |       |                         |       |       | cy<sup>b</sup> (Ef), % |
|-----|-----------------|------------------------------------|---------------------------|-------|------------------------|-------|-------|------------------------|
| Tests of FBD & IVM |                  |                                    |                           |       |                       |       |       |                          |
| 1   | Control         | Placebo                           | -                         | 20    | 85                     | -     | -     | -                      |
| 2   | Treatment       | FBD/IVM/PVP (1/1/9)                | IVM – 0.2, FBD – 3.0      | 10    | 0                      | 0     | 100   | 100 100 NA |
| 3   | Treatment       | FBD/PVP (1/9)                      | FBD – 3.0                 | 10    | 10                     | 1.70  | 88.3  | 99.7 92.1 < 0.001 |
| 4   | Treatment       | FBD substance                      | FBD – 3.0                 | 10    | 50                     | 210.4±78.8 0.23 | 41.2  | 67.7  | 17.3 > 0.05 |
| Tests of TBD & IVM |                  |                                    |                           |       |                       |       |       |                          |
| 5   | Control         | Placebo                           | -                         | 20    | 80                     | 484.2±77.2 2.69 | -     | -     | -                      |
| 6   | Treatment       | TBD/IVM/PVP (1/1/9)               | IVM – 0.2, TBD – 3.0      | 10    | 0                      | 0     | 100   | 100 100 NA |
| 7   | Treatment       | TBD/PVP (1/9)                     | TBD – 3.0                 | 10    | 60                     | 351.4±117.7 2.70 | 25.0  | 27.5  | 0 NA                   |
| 8   | Treatment       | TBD substance                     | TBD – 3.0                 | 10    | 80                     | 410.5±94.1 2.70 | 0     | 15.3  | 0 NA                   |

<sup>a</sup> In the numerator is the arithmetic mean, in the denominator is the geometric mean of the number e./ g.f.

<sup>b</sup> Geometric mean efficiency.

<sup>c</sup> Geometric mean values.

Statistically significant at P ≤ 0.05 when geometric means were compared to placebo.

NA – no statistical analysis was applied.

From the data of table 3 it follows that the substance FBD compared with its DSC (No. 2 and No.3), showed significantly less activity in gastrointestinal strongylatoses of sheep. At the same time during this invasion, the substance of TBD and its DSC (No. 6 and No. 7) were not effective.

When evaluating the effectivity of DSC based on FBD and IVM with sheep moniesiosis, it was found that, animals of the control group are infected by 30.0% with an average (arithmetic) value egg numbers 366.4 ± 134.5 instances (ins.) and the geometric mean - 3.10 e. / g.f. In the series of experiments with TBD and IVM, the sheep of the control group are infected with moniesia by 25.0% at the average number of eggs is 237.5 ± 105.7 ins. and the geometric mean – 2.90 e. / g.f. (see table 4, No. 1 and No. 5).

In the experimental group, when testing DSC of the composition FBD / IVM / PVP with moniesiosis (see table 4, No. 2) the indicator EE amounted to 66.7%, the indicator IE - 95.8%, decrease in geometric mean number of eggs (Ef) 29.7% at P> 0.05. DSC of composition FBD/PVP (No. 3) in the experiment was less active, EE was 66.7%, IE –87.8%, Ef 14.6% at P> 0.05. FBD substance
(No. 4), at a dose of 3.0 mg / kg b.w. was not active enough, the EE was 33.4%, IE - 60.3%, Ef - 6.5% at P> 0.05.

When testing the DSC of composition of TBD / IVM / PVP with moniesiosis in the experimental group (see table 4, No. 6), the rate of extensibility was 20.0%, the rate of intensity - 54.0%, decrease in geometric mean number of eggs (Ef) 6.9% at P> 0.05. DSC composition of TBD / PVP (No. 7) in the experiment also showed low activity, EE was 20.0%, IE - 47.7%, Ef - 3.5% at P> 0.05. Substance of TBD (No. 8) in a dosage of 3.0 mg / kg b.w. at moniesiosis turned out to be ineffective, EE indicators - 0%, IE - 13.5%, Ef -3.5% at P> 0.05.

| № | Group of animals | Preparation | Dose, mg of Al/kg b.w. | Number of sheep in group | EI, % | ANE*, e / g.f. | EE, % | IE, % | Efficacyb (Ef), % | p-Valuec |
|---|-----------------|-------------|------------------------|--------------------------|-------|---------------|-------|-------|-------------------|---------|
| 1 | Control         | Placebo     | -                      | 20                       | 30    | 366±134.5     | -     | -     | -                 | -       |
| 2 | Treatment       | TBD/IVM/PVP (1/1/9) | IVM – 0.2, TBD – 0.2 | 10                       | 10    | 15.3          | 2.18  | 66.7  | 95.8              | > 0.05  |
| 3 | Treatment       | TBD/IVM/PVP (1/1/9) | FBD – 3.0, TBD – 0.2   | 10                       | 10    | 44.6          | 2.65  | 66.7  | 87.8              | > 0.05  |
| 4 | Treatment       | TBD/IVM/PVP (1/1/9) | FBD – 3.0, TBD – 0.2   | 10                       | 20    | 145.5±98.7    | 2.90  | 33.4  | 60.3              | > 0.05  |
| 5 | Control         | Placebo     | -                      | 20                       | 25    | 237.5±105.7   | 2.90  | -     | -                 | -       |
| 6 | Treatment       | TBD/IVM/PVP (1/1/9) | IVM – 0.2, TBD – 0.2   | 10                       | 20    | 109±82.7      | 2.70  | 20.0  | 54.0              | > 0.05  |
| 7 | Treatment       | TBD/IVM/PVP (1/1/9) | TBD – 3.0, TBD – 0.2   | 10                       | 20    | 124.2±87.1    | 2.8   | 20.0  | 47.7              | > 0.05  |
| 8 | Treatment       | TBD/IVM/PVP (1/1/9) | TBD – 3.0, TBD – 0.2   | 10                       | 30    | 205.6±107.3   | 2.8   | 0     | 13.5              | > 0.05  |

*a In the numerator is the arithmetic mean, in the denominator is the geometric mean of the number e / g.f.

*b Geometric mean efficiency.

*c Geometric mean values.

Statistically significant at P ≤ 0.05 when geometric means were compared to placebo.
NA – no statistical analysis was applied.

From the data of table 4 it follows that the original substance FBD with moniesiosis of sheep showed significantly less activity compared to its DSC. At the same time, the substance of TBD and its DSC with this invasion were not effective.

In experiments (see table 5), when evaluating the effectiveness of DSC based on FBD and dicroceliosis, the control the sheep group was infected by 45.0% with an average number of eggs of 38.4 ± 11.6 ins. and the geometric mean - 1.90 e./ g.f. In this series of experiments, sheep the control group were infected with dicrocelia by 40.0% with an average number of eggs of 34.2 ± 10.2 ins. and the geometric mean - 1.90 e. / g.f. (see table 5, No. 1 and No. 5).

In the experimental group when testing the DSC of composition of FBD / IVM / PVP with dicroceliosis (see table 5, No. 2) the rate of extensibility was 55.6%, the rate of intensification– 64.9%, decrease in geometric mean number of eggs (Ef) 5.3% at P> 0.05.
DSC composition FBD / PVP (No. 3) in the experiment turned out to be less active, the EE rate was 33.4%, IE -56.3%, Ef - 10.6% at P> 0.05. The initial substance FBD (No. 4) did not show sufficient activity, EE was 33.4%, IE - 49.0%, Ef - 5.3% at P> 0.05.

DSC of the composition of TBD / IVM / PVP with dicroceliosis was quite active, in experimental group No. 6, the extensibility indicator was 75.0%, the intensification rate was 96.8%, a decrease in the geometric mean number of eggs (Ef) of 99.9% at P<0.001. DSC composition TBD / PVP ((No. 7) in the experiment also showed 100% activity. The substance of TBD (No. 8) in a dosage of 3.0 mg / kg b. w. with dicroceliosis was not active enough, the EE -50.0%, the IE was 76.6%, Ef -21.1% at P> 0.05.

Table 5. Efficiency of DSC parasitocides in sheep dicroceliosis.

| №  | Group of animals | Preparation | Dose, mg of AI /kg b.w | Number of sheep in group | EI, % | ANEa, e / g.f. | EE, % | IE, % | Efficacyb (EI, %) | p-Valuec |
|----|-----------------|-------------|------------------------|--------------------------|-------|----------------|-------|-------|------------------|----------|
| 1  | Control         | Placebo     | -                      | 20                       | 45    | 38.4±11.6      | -     | -     | -                | -        |
| 2  | Treatment       | FBD/IVM/ PVP (1/1/9) | IVM – 0.2, FBD – 3.0  | 10                       | 20    | 13.5±9.2       | 55.6  | 64.9  | 5.3              | > 0.05   |
| 3  | Treatment       | FBD/PVP (1/9) | FBD – 3.0              | 10                       | 30    | 16.8±9.0       | 33.4  | 56.3  | 10.6             | > 0.05   |
| 4  | Treatment       | FBD substance | FBD – 3.0              | 10                       | 30    | 19.6±10.4      | 33.4  | 49.0  | 5.3              | > 0.05   |
| 5  | Control         | Placebo     | -                      | 20                       | 40    | 34.2±10.2      | -     | -     | -                | -        |
| 6  | Treatment       | TBD/IVM/ PVP (1/1/9) | IVM – 0.2, TBD – 3.0  | 10                       | 10    | 1.1±0.4        | 75.0  | 96.8  | 99.9             | < 0.001  |
| 7  | Treatment       | TBD/PVP (1/9) | TBD – 3.0              | 10                       | 0     | 0               | 100   | 100   | 100              | NA       |
| 8  | Treatment       | TBD substance | TBD – 3.0              | 10                       | 20    | 8.0±6.7        | 50.0  | 76.6  | 21.1             | > 0.05   |

a In the numerator is the arithmetic mean, in the denominator is the geometric mean of the number e / g.f.
b Geometric mean efficiency
c Geometric mean values
Statistically significant at P ≤ 0.05 when geometric means were compared to placebo.
NA – no statistical analysis was applied.

From the data of table 5 it follows that in experiments with dyrococeliosis of sheep as the starting substance FBD and its DSC did not show sufficient activity. Whereas the TBD complexes at dicrocelial infestations were highly effective, while the initial substance of TBD in the experiment did not show high activity.

In a series of experiments to evaluate the effectivity of DSC based on FBD and IVM in melophagosis, the control group of sheep was infected at 95.0%, with an average value 19.9 ± 2.3 ins. and geometric mean 1.30 ins. per animal. Sheep control groups (based on FBD) are infected with bloodsuckers by 90.0% with an average value of 22.6 ± 2.9 ins and average geometric value 1.30 ins. parasite per animal (see table 6, No. 1 and No. 5).

In the experimental group, when testing the DSC of the composition FBD / IVM / PVP with melophagosis (see table 6, No. 2) the rate of extensibility was 79.0%, the rate of intensification - 92.5%, a decrease in the geometric mean number of bloodsuckers (Ef) by 38.5% (P> 0.05). DSC of composition of FBD / PVP (No. 3) was not effective, the EE was 15.8%, IE - 6.6%, Ef- 0%. The initial substance FBD (No. 4) also did not show activity, EE amounted to 5.3%, IE - 20.1%, Ef - 7.7% at P> 0.05.

DSC of TBD / IVM/ PVP composition during melophagosis was quite active in the experimental group No. 6, the indicator of extensibilit y was 67.7%, intensification - 96.1%, a decrease in the
From the data of table 6 it follows that in experiments with melophagosis of sheep as the starting substances FBD and TBD, and their DSC did not show activity. Active enough for melophagosis there were only DSC with the inclusion of IVM.

4. Discussion of the results
In Siberia and Gorny Altai, a parasitic complex parasitizes in sheep invertebrates, which is represented mainly by nematodes of the Strongylata suborder, flatworms of the genera Moniezia and Dicrocelium, insects sheep gadfly (Oestrus ovis L.) and sheep’s bloodsucker (Melophagus ovinus L.). From the standpoint of rational control in system of antiparasitic measures it is necessary to involve complex preparations corresponding spectrum of action in various combinations (nematocides, cestocides, trematocides and entomocides). In the experiments tested Mechanochemochemical compositions with PVP some drugs as IVM, FBD and TBD. In regulated by Manufacturers doses of IVM possesses high efficiency and a wide parasitocidal spectrum against nematodes and parasitic arthropods, FBD against nematodes and cestodes, TBD against trematode [8, 22, 23].

In the study of infection of animals with helminths, we used domestic method of quantitative counting of helminth eggs in samples of feces using VIGIS cameras, at the same time foreign researchers widely apply the technique McMaster (MAFF, 1986) [19]. Studies have not found a difference in effectivity methods of McMaster and Kotelnikov – Khrenov using the VIGIS counting chamber [24].
When assessing efficacy, it was used as a traditional method using arithmetic mean values of infection, and geometric mean, a combination of these approaches makes it possible to more fully judge the parasitocidal activity of drugs.

Antiparazitic developed by us using mechanochemical technology compositions allowed to significantly increase the solubility of drug substances. The increase in the solubility of SD based on the substances FBD, TBD, IVM and PVP polymer is an indicator of the formation of the corresponding supramolecular complexes. Like it was expected that an increase in the solubility of substances led to an increase in bioavailability and the efficacy of the resulting parasitocidal agents. Conducted experiments in sufficient least confirmed this pattern.

So, the minimum regulated dose of oral FBD and TBD for various helminthiases of sheep are 10 mg per kg of animal weight [23]. In the experiments more than 3 times reduced doses of FBD and TBD (3 mg / kg b.w.) in DSC compositions with PVP showed quite high efficiency with helminthiases.

DSC of the composition FBD / IVM / PVP (1/1/9) showed high efficiency against gastrointestinal strongylates and moniesia (IE 100 and 95.8%, Ef 100 and 29.7%, respectively), against sheep bloodsucker (IE 92.5%, Ef - 38.5%) and is ineffective against wild-goats. DSC composition FBD / PVP was quite active against gastrointestinal strongylates and moniesia (IE - 99.7 and 87.8%), but not active against wild goats and bloodsuckers. DSC of composition of TBD / IVM / PVP (1/1/9) showed high efficacy against gastrointestinal strongylates (action of ivermectin) and dicrocelium (IE – 100 and 96.8%, Ef - 100 and 99.9%) and was not effective against moniesia and sheep bloodsuckers.

DSC of composition of TBD / PVP was highly effective against wild-boar (100%), but not active against gastrointestinal strongylates, moniesia and bloodsucker. In all experiments the initial substances FBD and TBD showed significantly less activity. Similar results in assessing the activity of mechanically modified preparations of FBD and TBD with helminthiases of sheep and cattle are shown in other works. So, with oral administration of a complex based on FBD and PVP with the addition of 1-2% sodium dioctyl sulfosuccinate demonstrated high drug efficacy in doses of 2.0 and 3.0 mg / kg b. w. by AI (92.4-100%) against gastrointestinal strongylates and moniesia in sheep [25]. The 100% effectiveness of the TBD complex (triclabendazid) is shown with fascioliasis of cattle in a dose of 2.5 mg / kg individually in the form of an aqueous solution orally and in a production experiment at a dose of 3.0 mg / kg mixed with food at a dose of 5 times reduced compared with the original substance of the drug [26]. As a result, we can conclude that the developed antiparasitic compositions based on SD of fenbendazole, triclabendazole and ivermectin are highly effective against strictilatoses of the gastrointestinal tract, moniesiosis and dicroceliosis of sheep and in sufficiently reduce the number of parasitic bloodsuckers. Application of these compositions will sufficiently control the incidence of sheep basic parasitic infestations in Siberia.

5. Conclusions
The study demonstrated the high efficiency of SD composition FBD / IVM / PVP (1/1/9) in a dose of FBD - 3.0, IVM - 0.2 mg / kg b. w. when administered orally against gastrointestinal strongylates, moniesia and bloodsucker in sheep. SD composition TBD / IVM / PVP in a dose of TBD - 3.0, IVM - 0.2 showed high efficiency against gastrointestinal strongylates and dicrocelia and it was not effective against moniesia and sheep bloodsuckers. Starting materials (FBD and TBD) showed significantly less activity in parasitic infestations of sheep.

The high parasitocidal activity of the studied SD and their complexes is explained by increased solubility in water and bioavailability. The use of such compositions suggests the possibility of a significant (three-fold) reduction in the dosage of FBD and TBD without loss parasiticidal activity.

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