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Original Article

Development of Resource-Saving Biotechnology for the Production of a Feed Additive from Distiller's Wastes with Probiotic Properties

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Abstract

The trend towards an increase in ethanol production on a global scale and the tightening of restrictive measures regarding the disposal of by-products from production increases the relevance of research in finding ways to process them. This study aimed to assess the effectiveness of a feed additive containing dried stillage fermented with pre-immobilized cultures of *Lactobacillus pontis 67, Lb. casei 22, Lb. paracasei 104* when finishing steers. The dose of inoculum (3.0%) of a liquid probiotic drug based on the consortium *Lb. pontis 67, Lb. casei 22, Lb. paracasei 104* was determined, cultivation time 24-30 hours at a bacterial titer of 10-10 CFU/ml, and the characteristics of fermented stillage and a feed additive based on it were established. Studies were conducted to assess the effectiveness of finishing when using fermented wheat stillage on the qualitative characteristics of the carcass of 13-month-old Kazakh white-headed steers on finishing. The studies were conducted in 2 groups: control and experimental (13% fermented stillage). The experimental group showed higher indicators of average daily gain ($P<0.05$), carcass weight, and slaughter yield ($P<0.05$). According to the results of the conducted research, it can be concluded that the replacement of protein components of compound feed with stillage in an amount of 10% and a probiotic drug based on the bacteria *Lb. pontis 67, Lb. casei 22, Lb. paracasei 104* in an amount of 3.0% contributes to an increase in weight gain when finishing steers by 9.1±0.3 kg, average daily gain by 417±2.0 g, and slaughter yield by 3.1±0.2% compared with the control group ($P<0.05$).

Keywords: Stillage, Feed additives, Compound feed, Probiotic, Feedlot steers

1. Introduction

The production of ethanol and bioethanol is an important and rapidly growing market sector. Every year, the range of raw materials for the production of ethanol is expanding with the addition of new sources: corn (1), sugar cane (2), sugar beet (3), and wasted bread (1). At the same time, a significant part of the bioethanol by-products is stillage. Approximately 13 gallons of stillage remain during the production of 1 gallon of bioethanol from starch-containing substrates (4).

The tendency to increase production on a global scale and the tightening of restrictive measures regarding the disposal of by-products of ethanol and bioethanol production increases the relevance of research in the search for new ways of processing. Grain stillage is a nutritious product for fattening livestock, but at the same time, it is a watery feed that spoils with improper storage and use. Thus, on the one hand, distiller's grains are a distiller's waste, which can pollute the environment; therefore, dumping distiller's grains into reservoirs or sewers without preliminary processing is prohibited by the Law of the Republic of Kazakhstan and therefore, for this reason, requiring mandatory recycling. On the other hand, distiller grains, due to their high protein content, vitamins, and minerals, can be valuable raw materials for processing for various purposes (5).

There is evidence of positive results when using post-distillery waste in various forms in cattle (6), sheep (7), broiler chickens (8), and fish (9).

It is also known about positive results when using various types of the liquid part of the stillage for fermentation and production of lactobacillus biomass for use in the production of lactic acid (1, 3). The protein and nitrogen content in the composition of stillage allows for high growth and density (3) of a number of lactobacillus strains; calcium, magnesium, manganese, and other metals in the composition of stillage stimulate the growth of Lactobacillus (10). The positive effect of fermented animal feed has been shown to have a positive effect on a number of indicators, such as a low pH level and, accordingly, an increase in the shelf life and improved digestibility of animal feed (11), as a result of which the productivity of animals can increase, the safety of livestock and the efficiency of livestock production, in general, can increase.

One of the main conditions for the effectiveness of fermented livestock feed is a high level of viable microorganisms in the feed, at least 10^6 -10^9 CFU kg^{-1} (12). In connection with the above, the use of stillage together with various strains of lactobacilli may be promising.

In 2006, the European Union imposed a ban on antibiotics as growth stimulants in animals due to the danger of developing resistance to pathogenic microorganisms with constant use in diets. In this regard, numerous studies have been conducted on the effect of antibiotics on the human body when eating meat, dairy, and egg products (13).

Studies on replacing antibiotics with drugs safe for animal and human health and research on the development of technology for safe products are becoming increasingly priorities. One of the alternative solutions to this problem may be using probiotics and products derived from them (14).

This study aims to develop a resource-saving technology for producing a feed additive from distiller waste with probiotic properties.

2. Materials and Methods

2.1. Preparation and Immobilization of a Liquid Probiotic Drug

Consortium of highly active cultures of lactic acid bacteria with collection numbers – (B-449) *Lb. pontis 67*, (B-7) *Lb. casei 22*, (B-446) *Lb. paracasei 104*, deposited in the collection of cultures of "KazRI Processing and Food Industry" LLP (KazRIPFI LLP), Republic of Kazakhstan, Almaty, in the form of dry powder, was reactivated by adding 50 ml of MRS medium (HiMedia, India) to a 100 ml flask under sterile conditions in the ratio of strains 1:1:1 (5.0%). The flask was incubated at 37.0 °C for 24 hours.

To obtain a liquid probiotic drug, a reactivated consortium of lactic acid bacteria is sown in an amount of 5% in the following nutrient medium: a mixture of wheat and soy flour + water (hydro module 1:1.5) + 0.1% sorbent (modified high-carbon shungite/amorphous silicon in chelated form); cultivation is conducted at a temperature of 35±2.0 °C for 22±2.0 hours (15).

Silicon chelate was used at “Mechcenter” LLC, Shungite chips (“Argo” company, “Zazhoginskoye” deposit, Russia) were pre-sieved through a filter with a cell diameter of 0.5 mm to obtain a more constant fraction in size. Then the shungite was washed with distilled water and sterilized in an autoclave at 1 atmosphere for 30 minutes.

2.2. Stillage Preparation

Wheat stillage was purchased in “Talgar Alcohol” LLP (Talgar, Republic of Kazakhstan).

We received canned stillage with a probiotic in two stages: concentration and fermentation of the distiller’s grains.

The concentration of the distiller’s grains was conducted using a technology similar to that used in industrial processing of distiller’s grains when hot distiller’s grains are fed into a decanter centrifuge and are divided into a precipitate and a liquid part. The sediment contains a pellet and most of the alcoholic yeast, and the liquid part contains the distiller's yeast and dissolved substances.

Under laboratory conditions, we obtained condensed distiller’s grains as follows:

- the pellet was separated on a sieve with a pore size of about 1.0 mm;
- the filtrate was defended by the distiller's grains, and the filler liquid was used for disposal;
- part of the resulting loose sediment was centrifuged, and the fugate was going to be disposed of;
- all parts were connected (shot, loose sediment, centrifuge sediment), and a distiller's grains concentrate with the consistency of liquid sour cream was obtained. Anaerobic cultivation of lactic acid bacteria is possible in such a substrate.

2.3. Production of Dry Preparation

In the further production of the dry preparation, the method of immobilization (contact-sorption dehydration) of the liquid preparation on wheat bran is used: hydro module - 1:1. Mixing of the components is carried out in a mixer "SM-150". The wet mixture is laid out on the trays of the RT-CD dryer and dried at a drying temperature of 55.0 °C; the drying duration is 8 hours until the absolute humidity is 7-8%. The shelf life of the dry preparation is 4 months at 4-6 °C (15).

2.4. Preparation of Feed Additives and Compound Feed Formula

The program "AMTC Cattle Pro," based on the recommendations of the National Research NRC (16), was used to prepare the diet and formulae for compound feeds and feed additives. The feed components for both groups of animals were weighed according to the formula and mixed in a vertical mixer "VM-5S".

2.5. Scientific and Economic Experiments

To conduct scientific and economic experiments on finishing steers in economic conditions, two groups (control and experimental) on the principle of analogs, 5 livestock at the age of 13 months each consisting of Kazakh white-headed steers.

The duration of the finishing experiments was 22 days. The experiments were conducted in the conditions of a peasant farm, “Sapaly Kesek” LLP (Kaskelen, Republic of Kazakhstan).

When feeding the feedlot steers of the control group, a diet operating on a peasant farm was used (hay, grain mixtures crushed according to the norms of the feeding diet for 1 animal per day). For the steers of the experimental group, the following methods were used: hay, compound feed based on a feed additive. All animals had unlimited *ad libitum* access to water and feed. Feeding was carried out twice a day at 8.00 and 16.00 daily. Before setting the steers for feeding, an individual weighing was performed. During the experiment, the provision of hay, grain fodder (wheat, barley, etc.), compound feed, and watering were strictly controlled.

Feed consumption was determined by weighing the feed and feed residues before feeding.

The pre-slaughter exposure of the steers was 24 hours; the animals were kept in a shed with a temperature of 22.0 ˚C, without feed, but with free access to water; the density of the animals was no more than 2.0 m^2. Before the slaughter, the animals were cleaned and washed.

The slaughter of animals was carried out in the slaughterhouse of the peasant farm "Sapaly Kesek" LLP; the animal carcasses were divided according to the corresponding Republic of Kazakhstan standard UNECE, 2012).

2.6. Analytical Methods

The titer of lactic acid bacteria (CFU/ml) was determined by the method of limit dilutions followed by seeding into semi-liquid nutrient media. From dilutions of the drug 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9}, 10^{-10}, the degree of which depends on the amount of CFU in the test sample, 1 ml of microbial suspension is sown in the test tubes containing 9.0 ml of semi-liquid nutrient medium. Dilution of 10^{-6} of the drug in 0.9 % sodium chloride solution corresponds to a dilution of 10^{-6} in a nutrient semi-liquid medium. The inoculation is incubated at a temperature of 37°C, depending on the type of microorganism. Incubation is carried out for 24-72 hours, depending on the growth rate of the colonies. At the end of incubation, dilutions are noted in which there is the growth of typical colonies for this type of microorganisms; in these dilutions, the colony is counted.

The dry matter content was determined by the method based on drying a product sample in a drying cupboard in a weighing bottle at a temperature of 105 ˚C to a constant mass (17), and the fiber content was determined by the Scharrer-Kurschner method (18), calcium and phosphorus on an atomic absorption spectrophotometer (AAS-3, Germany) (BS EN 13805:2014, 2014).

The protein content was determined by the Kjeldahl method by calculating total nitrogen and multiplying by a coefficient of 6.25, crude fat was determined by the Soxhlet method (17), and the pH value was determined by the CP-315 pH meter.

2.7. Statistical Analyses

The experiments were carried out in three-fold repetition. For all measurements, the values are indicated by ± standard deviation. The differences in the measurements of the experimental and control groups were calculated using the one-way ANOVA analysis using the Tukey test. The measurement value $P<0.05$ was taken into account as significant.

3. Results

Studies have been conducted to determine the parameters of the immobilization of a consortium of lactic acid bacteria (LAB) and to obtain a condensed stillage. Studies have also shown good survival with the improvisation of the condition in vivo of the gastric tract of animals (15). A pre-prepared liquid immobilized probiotic drug consisting of a reactivated consortium of bacteria *Lb. pontis 67, Lb. casei 22, Lb. paracasei 104,* mixtures of wheat flour, soy flour, and water were used to determine the dose of seed material for fermentation of condensed stillage (Table 1). In plastic bottles with tightly closed lids, fermentation of the LAB distiller's grains was carried out at 35 °C. The bottles were sterilized with ethyl alcohol. An immobilized probiotic drug of 1-5% was introduced into the post-stillage concentrated on a decanter centrifuge. During anaerobic cultivation, one culture liquid was shaken once every 8 hours. Before seeding, the pH of the concentrate was adjusted to 6.0 using 0.1 M sodium hydroxide (NaOH).

Table 1. The effect of the dose of seed material on the LAB cultivation

Dose of seed material, %	LAB titer, CFU/ml		
	24 h	30 h	48 h
1	10^9	10^9	10^{10}
3	10^9	10^{10}	10^{10}
5	10^{10}	10^{10}	10^{10}

At a dose of 3.0% seed material, the titer of the LAB consortium does not have time to reach the values of

10^{10} CFU/ml by 24 h of growth. Taking into account that in production conditions, the packing time of canned distiller's grains, the waiting time for sending to the consumer, and the transportation time will be significant, it can be assumed that the dose of the seed material of 3.0% and the cultivation time of 24-30 hours will be sufficient.

Further, the main characteristics of a sample of canned distiller grains with a probiotic drug content of 3.0% were determined (Table 2).

Table 2. Characteristics of fermented distiller's grains (with the introduction of a 3.0% probiotic drug)

Indicators	Value
pH	4.22
Titrated acidity, ˚H	14.5
Cell titer, CFU/ml	10^{10}
Dry matter, %	15.30
Crude protein, % on absolute dry matter	41.89
Nitrogen (N), %	6.70
Fiber, % on absolute dry matter	1.43
Fiber, % on fresh weight	0.22

We have previously studied wheat stillage's chemical composition, nutritional, and energy value. Studies have shown that stillage has a relatively high content of protein and a number of trace elements and is a fairly valuable secondary raw material, and this allowed the selection of the component composition for the development of a feed additive. As can be seen from table 2, the titer of LAB in canned distiller's grains is 10^{-10} CFU/ml; in the content of dry matter in liquid alcohol compared to previous experiments, an increase of almost 2 times is noticeable: 15.3% and 7.5%, respectively, which also helps to reduce transportation costs. There is also a noticeable increase in the proportion of protein from 32.5% to 41.89%.

The fermented distiller's grains were mixed with wheat bran in a ratio of 1:1 and dried at a temperature of 55.0℃ to an absolute humidity of 7-8%. The dry mixture was later used as part of a feed additive; the rate of its introduction was 13.0% (3.0% probiotic drug).

The composition of the developed feed additive is presented in table 3.

Table 3. Composition and nutritional value of a feed additive from alcohol production waste with the introduction of a probiotic drug

Name of the component	Component content, %
Fermented stillage (in dry form)*	13.0
Soy meal	40.0
Wheat germ	16.0
Wheat bran	20.0
Stern chalk	5.0
Table salt	3.0
Premix **	3.0
Total:	100.0
Content (in 1 kg)	
Exchange energy (MJ/kg)[a]	9.22
Dry matter, (g/kg)[b]	9.36
Crude protein, (g/kg)[b]	160
Digested protein, (g/kg) [a]	122
Crude fat, (g/kg)[b]	37.5
Calcium, (g/kg)[b]	8.2
Phosphorus, (g/kg)[b]	9.8
Titer of probiotic LAB, CFU/g[b]	1×10^{-9}

A - determined by calculation. b - determined analytically. * 10% - fermented stillage (in dry form), 3% - probiotic drug (excluding wheat bran). ** At least 20.3 g of calcium, 29.3 g of phosphorus, 0.042 g of copper, 0.02 g of cobalt, 0.05 million cubic meters of vitamin A, 0.01 million cubic meters of vitamin D, 0.4 thousand cubic meters of vitamin E, barley turf -0.61 kg, urea - 0.15 kg, monocalcium phosphate -0.12 kg, molasses -0.08 kg per 1 kg of premix.

The analysis of the chemical composition, the content of metabolic energy, and the titer of lactic acid bacteria in the developed pilot batch of feed additives indicates that it is a source of protein, fat, and energy (Table 3). The titer of probiotic bacteria in the finished feed additive after drying and mixing decreased slightly while maintaining a relatively high level of live LAB.

Further, the feed additive was used as part of compound feed for partial replacement of protein raw materials (Table 4).

Introducing feed additives from distiller waste into compound feed did not have a noticeable effect on quality indicators. Crude fat, crude fiber, etc., is at the level of control samples. The live probiotic lactic acid bacteria titer in the developed compound feed was $9x10^6$ CFU /ml.

In the peasant farm "Sapaly Kesek" LLP in Kaskelen, the Republic of Kazakhstan, scientific and economic experiments were conducted on finishing steers with compound feeds developed based on feed additives (Table 5).

The diet composition of the control group of animals included compound feed and haylage. The diet composition of the experimental group of animals included compound feed with a feed additive and haylage.

When feeding at the beginning of the experience was not a significant difference in live weight of the experimental and control groups ($P>0.05$); by the end of the feeding period (22 days), steers of the experimental group had significant weight gain compared to control group animals ($P<0.05$).

Scientific and economic experience of feeding showed that the use of feed with the use of stillage and probiotic strains for feedlot calves in the experimental group had improved the average daily gain of live weight of calves at 417±2.0 g in comparison with the control group.

After feeding, a controlled slaughter of steers was carried out (Table 6).

Table 4. Composition and nutritional value of compound feeds based on feed additives for feedlot bulls

Name of the component	Content of compound feed components, %	
	Control	Experiment
Barley	20.0	25.0
Corn	10.0	-
Feed wheat	20.0	25.0
Wheat bran	31.0	28.0
Feed additive	-	22.0
Rapeseed meal	15.0	-
Feed phosphate*	2.0	-
Table salt	1.0	-
Premix **	1.0	-
Total:	100.0	100.0
Content (in 1 кг)		
Exchange energy (MJ/kg)[a]	9.25	9.26
Dry matter, (g/kg)[b]	939	939
Crude protein, (g/kg)[b]	162	164
Digested protein, (g/kg) [a]	125	126
Crude fat, (g/kg)[b]	37.8	37.9
Crude fiber, (g/kg)[b]	98.6	98.7
Calcium, (g/kg)[b]	8.5	8.7
Phosphorus, (g/kg)[b]	10.0	10.1

a - determined by calculation. b - determined analytically. * Contains at least 34% calcium and 18% phosphorus. ** At least 0.042 g of copper, 0.02 g of cobalt, 0.05 million cubic meters of vitamin A, 0.01 million cubic meters of vitamin D, 0.4 thousand cubic meters of vitamin E, barley turf - 0.61 kg, urea - 0.15 kg, molasses - 0.08 kg per 1 kg of premix

Table 5. Experience in feeding calves (13 months)

Parameters	Groups		Significance
	Control	Experimental	
Number of animals (n)	5	5	-
The initial average weight of 1 animal, kg	338.6±4.6	346.0±4.49	not significant
The average weight of 1 animal at the end of the experiment kg	355.1±7.84	371.6±6.90	*
Weight gain of 1 animal, at the end of the experience, kg	16.5±1.45	25.6±3.36	*
Average daily gain, g	748±2.7	1165±2.5	*

Table 6. Results of the controlled slaughter of steers

Indicator	Group		Significance
	Control	Experimental	
Number of animals (n)	5	5	-
Carcass weight, kg	194.6±3.39	215.2±3.04	*
Slaughter yield, %	54.8±0.33	57.9±1.34	*

The values indicated are ± standard deviation calculated from three parallel measurements. * $P<0.05$, significant, not significant ($P>0.05$)

The measurement of carcass weight and slaughter yield in the experimental group showed higher results compared to the control group ($P<0.05$).

4. Discussion

The studies were conducted to assess the effectiveness of pre-fermented LAB wheat stillage in finishing livestock. The conducted cultivation of LAB at doses of seed material 1-3% showed relatively consistently high indicators in the substrate based on post-stillage (10^9-10^{10}), which is consistent with studies on the positive effect of stillage on the growth of *L. Plantarum* (3) and the growth of *L. rhamnosus* (1).

The result of finishing with a diet with fermented stillage in the experimental and control groups of steers showed an absolute increase in live weight of 16.5±1.45 and 25.6±3.36 kg, respectively. There is evidence of an undetected effect on the weight gain of Holstein steers when including a small volume of dried distiller's grains plus soluble (DDGS) (10%) in the diet (19). There is also evidence that 30% of DDGS did not affect the final weight when finishing steers (20).

However, a number of studies have noted a positive effect of the inclusion of wet distiller's grains plus soluble (WDGS) in the diet in the amount of 15% of the diet on the average daily gain ($P<0.01$) of crossbred Angus steers. There was also a maximum increase in the average daily gain ($P<0.01$) of feedlot steers when combined with WDGS with microbiological additives (NovaCell) (21). An increase in the weight gain of cattle (approximately 2.5%) was achieved with the use of lactate-utilizing and lactate-producing bacteria in the diet (22). Therefore, the use of probiotic drugs allows a more pronounced effect on the productivity of weight gain even when using small amounts of stillage, WDGS, and DDGS.

Analyzing the results of the experiments, we can conclude that the feed additive in the amount of 22%, containing dried fermented stillage (10%) with pre-immobilized cultures *Lb. pontis 67, Lb. casei 22, Lb. paracasei 104* (3%) feeding feedlot steers can partially be used to replace expensive protein raw materials partially. Using a probiotic drug improves the quality and sanitary condition of the feed additive, increasing the shelf life of their storage up to 4 months.

Joint use of stillage and the consortium *Lb. pontis 67, Lb. casei 22, Lb. paracasei* 104 allowed to obtain higher results in weight gain (by 9.1±0.3 kg), average daily gain (by 417±2.0 g), and slaughter yield (by 3.1±0.2%) of feedlot steers compared to the control group ($P>0.05$).

According to the results of the conducted research, the following conclusions can be drawn:

1. The chemical composition, nutritional, and energy values of stillage have been studied. The analysis of the experiments showed that in terms of the content of fiber, carbohydrates, protein, vitamins, trace elements, and nutritional value, stillage is a reasonably valuable secondary raw material and can be used as a component composition for the development of a feed additive.

2. The norms of stillage and probiotic drug as part of a feed additive for feedlot steers were determined: canned stillage with probiotic - 13.0%; probiotic drug with immobilized cultures: *Lb. pontis 67, Lb. casei 22, Lb. paracasei 104* - 3.0%.

3. A formula has been developed for a feed additive using stillage and a probiotic drug for feedlot steers based on distiller's waste with the introduction of a probiotic drug containing lactic acid bacteria, which allows replacing the meal in the feed, reducing its cost, as well as improving the microflora of the gastrointestinal tract of animals.

4. A scientifically-based formula for compound feed based on feed additives for feedlot steers has been developed.

5. In the feed mill of "KazRIPFI" LLP, a pilot batch of compound feed was produced based on the developed feed additive for feedlot steers. The chemical composition, nutritional value, and energy value (protein, fat, fiber, calcium, phosphorus, etc.) of the compound feed produced by the pair, with the introduction of a feed additive for feedlot steers, were studied. It is shown that compound feed is a feed product with a titer of live lactic acid bacteria of $9x10^6$ CFU/ml, balanced in protein content and other nutrients that meet the general requirements of the physiological characteristics of feedlot steers.

Scientific and economic experiments were conducted on feeding feedlot steers with experimental and control batches of compound feeds in the economic conditions of the peasant farm "Sapaly Kesek" LLP (Kaskelen, Republic of Kazakhstan). The use of therapeutic and preventive compound feed for feedlot steers in the experimental group allowed us to obtain higher results in weight gain (by 9.1±0.3 kg), the average daily gain (by 417±2.0 g), and in the slaughter yield (by 3.1±0.2%) of feedlot steers compared to the control group (P>0.05).

Authors' Contribution

Study concept and design: Z. A.
Acquisition of data: T. Z.
Analysis and interpretation of data: M. V.
Drafting of the manuscript: K. S.
Critical revision of the manuscript for important intellectual content: I. P.
Statistical analysis: S. K.
Administrative, technical, and material support: T. Z.

Ethics

All animal experiments were conducted in accordance with EU Directive 2010/63/EU for animal experiments.

Conflict of Interest

The authors declare that they have no conflict of interest.

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