E3S Web of Conferences 393, 03020 (2023) https://doi.org/10.1051/e3sconf/202339303020
EPPCT 2023

Experimental design study on the antimicrobial effect of compound herbs on poultry manure fed to ruminants.

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Abstract: In this paper, an experimental scheme based on poultry manure as a feed additive for ruminants along with a compound herbal medicine as a bacterial inhibitor of poultry manure was proposed by implementing linear simulations by polynomial cubic fitting method on today's topical environmental pollution issues of chicken manure and straw. The results of the fitting yielded that the herbal medicine addition at 1% was the best ratio value to effectively inhibit the bacteria of poultry manure and promote the growth of cattle. Therefore, this herbal dosing is effective for feeding ruminants. It also provides a new scientific research method for the application of poultry manure in ruminant feeding today.

1. Introduction

Previously, with the gradual rise in the standard of living of human beings, the increase in the number of the world population has not only led to a great increase in the demand for crops, but also the demand for poultry meat has likewise shown an upward trend, thus stimulating the rapid development of poultry farming and crop cultivation. We take corn crop production as an example, the survey found that between 1961 and 2020, world corn production has been a linear growth trend; currently the United States is the world's largest corn producer, with a production of more than 360.25 million tons in 2020, China and the European Union (27, FAO) is also a major area of corn production, production output of 260.67 million tons and 67.84 million tons, respectively; at the same time , Ukraine and Brazil are also a major corn crop producer, with production of 30.29 million tons and 103.96 million tons, respectively, in 2020. On the other hand, global poultry meat production because of the increase in the world's population in the past 50 years has also grown rapidly, between 1961 and 2014, an increase of nearly 12 times; the United States, as the world's largest producer of poultry, a single 2014 stocking production has exceeded 20 million tons, however, China and Brazil, stocking 18 million tons and 13 million tons, respectively; also followed closely behind. For more details, see - Figures I and II of the company survey data [1,2]. However, the rapid development of poultry farming, and cultivation brings food, meat and egg products, but also a large amount of poultry manure waste and waste crop straw. Investigation and research found that China, as the main production area of poultry farming, produced about 2.257 billion tons of livestock manure and urine according to the Sustainable Development Research Group of the Industrial Economics Research Office of the National Egg Industry Technology System of China [3]. It was found that the average annual area planted with corn in China reached 0.36 billion hectares, and the average annual production of corn straw reached 254 million tons [4]. However, in the rapid development of the world economy in recent decades, the output of a large amount of poultry manure and the crop production waste-corn straw has also brought a corresponding environmental pollution to the world. Therefore, the environmental problems caused by chicken manure and straw are becoming increasingly serious and have become a hot issue that needs to be addressed globally today.

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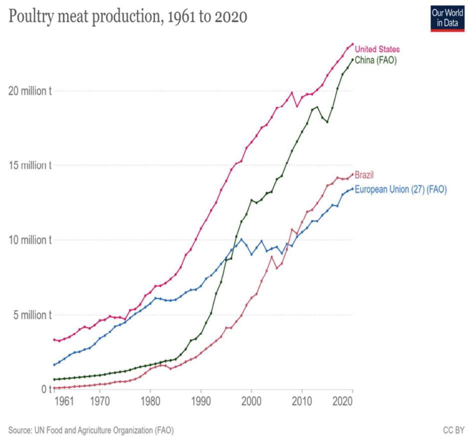

Figure 1. Statistical distribution of annual pollutant survey statistics by country.

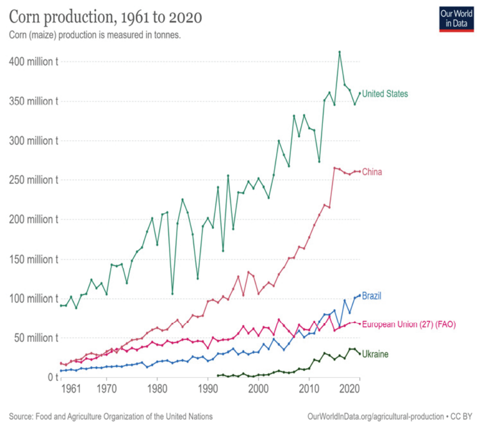

Figure 2. Scatter map of waste straw poultry stock surveys by country.

According to the analysis of the above graph, it can be concluded that Europe is a major poultry meat producing region with a poultry stock production of about 19 million tons in 2014, which is slightly lower than that of the United States. And the purpose of this paper is to study the relationship between poultry stock production and poultry manure in the European region to facilitate feeding studies using herbs and poultry protein additives in ruminants to better address the basic ecological issues. To explore how to add herbs and the amount of chicken manure admixture, the authors proposed an experimental protocol for the relevant study and used pyhton to perform regression analysis, which yielded the corresponding regression results and determined that 1% addition of herbs is the optimal equivalence value.

On the important issue of the important safety hazards posed by poultry manure in farm production, we have studied a large body of literature and have made a positive discussion of the literature. With reference to the results of the literature discussion we found that inappropriate handling or disposal of poultry manure poses risks to the ecosystem environment and human health. Poultry manure is contaminated with pathogenic microorganisms such as bacteria, fungi, viruses, parasitic protozoa, and helminths; antibiotics with drug-resistant genes and pathogenic microorganisms; heavy metals; growth and sex hormones such as estrogens, especially 17β-estradiol and testosterone; and pesticides such as dioxins, furans, PCBs, and polycyclic aromatic hydrocarbons[5]; and, common odor compounds in poultry manure are ammonia, dimethylamine (DMA), and trimethylamine (TMA), and studies on the genotoxic and cytotoxic activities of these compounds in model chicken cell lines (LMH) have shown that these compounds can induce cell death through necrosis and apoptosis[6]. If poultry manure is dispersed near rivers, lakes, and groundwater-rich areas, then the nutrients, organic matter, and pathogens in poultry manure will cause groundwater contamination, which in turn will affect drinking water safety and even endanger human health. By the same token, it follows that if poultry manure is used on protein additives for ruminants, there will also be a corresponding danger to them. Therefore, we propose the addition of appropriate amounts of herbs to inhibit the development of these bacteria. Current research on the sustainable use of poultry manure is still a hot topic in ecological science research today, and the main application areas include biomass energy, biogas production, and fertilization of chicken manure. Examples include: poultry biochar and unmodified manure to improve maize seed yield [7]; removal of nitrogen from anaerobic digestion of chicken manure to enhance biomethanation [8]; inoculation of microorganisms to compost chicken manure and maize straw to fertilize chicken manure[9]. However, chicken manure as a protein additive and Chinese herbs as bacterial-like inhibitors have been little studied by scientists. Therefore, the scientific research nature of this experiment is very necessary.

2. A review of the relevant literature on experimental setup methods.

The nutritional value of poultry manure as a ruminant feed additive is of considerable value. Due to the special digestive physiological structure of poultry, their intestinal tract is short, their ability to digest and absorb feed is poor, and there is a large amount of undigested grains and available nutrients in the excreted manure; moreover, poultry manure is rich in a high percentage of non-protein nitrogen, fiber and minerals, and they are characterized by a low energy value, and their use as feed applications than as fertilizer or methane production. On the other hand, the possibility of contamination by pathogens, mainly Salmonella, needs to be taken into account when considering its application as a feed additive, and in this regard, suggestions from different literature sources suggest the possibility of silage of this material type and that the silage method has the following advantages: low cost, reduction of potentially pathogenic microorganisms, better palatability, and reduction of undesirable taste [10]. Thus, the chemical composition of poultry manure is considered as a source of nutrients and bioactive substances, able to apply them in ruminant feeding.

Corn straw is an important source of roughage for ruminant livestock. Corn straw is rich in nutrients and contains many elements, including 44.22% carbon, 0.62% nitrogen, 0.25% phosphorus and 1.44% potassium, as well as calcium, magnesium and sulfur, and an average of 15% organic matter, making it a valuable source of renewable energy [11]. However, the crude protein (CP) and crude fat (EE) content of maize straw is low and does not meet the needs of ruminants well [12,13]. Maize straw not only affects palatability due to its high lignin content, but also affects the absorption and transformation of straw nutrients by animals, which limits the utilization of maize straw. The nutritional value and palatability of straw will be greatly improved after proper physical, chemical and biological treatment of the straw [14]. Corn straw has a high content of soluble carbohydrates and fibrous material, which can provide sufficient substrate for microorganisms and enzymes, so corn straw silage fermentation is a good choice.

MDK Jamee et al[15] used caged laying hen manure as the basis for silage with corn straw and molasses, and placed chopped corn straw in airtight plastic containers and treated with T0 (0% chicken manure), T1 (20% chicken manure), T2 (40% chicken manure) and T3 (60% chicken manure) at room temperature for 0, 30, 60 and 90 d to determine the physical quality, Obydul Islam et al[16] also used cage layer manure as a basis with rice straw (H.M. Saleh et al[17] used poultry manure as a dietary supplement in ruminant diets, which was found to be effective in reducing cost, reducing protein deficiency in the diet and solving disposal problems. protein deficiency in diets and solving disposal problems with considerable effect.

As a type of ruminant livestock, while using poultry manure and corn straw as ruminant feed sources, it is still necessary to consider the diseases that may be caused by harmful components in poultry manure, including beef toxigenic bacillus poisoning, bovine rumen acidosis and many types of bovine diarrhea. For example, the possible presence of botulinum toxin-producing Clostridium perfringens in poultry manure when it is poorly silenced or even fails may lead to a large outbreak of beef toxin poisoning; and, carrion formed from the death of birds and poultry is likewise considered a possible source of type D beef toxin poisoning; similarly, large amounts of grass silage may be contaminated with type D/C botulinum spores, leading to an outbreak of beef toxin outbreak of Clostridium botulinum poisoning[18]. The rationing of concentrate, roughage, and other feed additives in the diet should be considered when feeding cattle for production. Ruminal acidosis results when cattle on the diet consume excessive amounts of fermentable carbohydrates, resulting in the accumulation of nonphysiological acids in the rumen along with a decrease in ph[19].

The combination of Pulsatilla prescription has strong killing and inhibiting effects on a variety of germs, and animal experiments have shown that: it has obvious anti-inflammatory and ulcer healing effects on experimental animals; it can regulate a variety of immune cytokines and promote immune function; it can significantly inhibit the movement of the intestinal tube of isolated experimental animals, anti-diarrhea, and anti-endotoxin damage to the organism.[20,21]. Dandelion is a genus of dandelion plants widely distributed in the warm temperate regions of the northern hemisphere [22]. As an edible herb of plant origin, this genus is mainly used as an anti-inflammatory, antibacterial, anti-allergic, choleretic and antioxidant agent because of its bioactive metabolites such as phenolic compounds, sesquiterpene lactones, polysaccharides and flavonoids [23]. According to investigations, dandelion affects rumen microorganisms and metabolites, thus enhancing rumen fermentation in lactating cows [24]. Licorice (Glycyrrhiza uralensis Fisch) is likewise a traditional medicinal herb in Chinese medicine, it is a leguminous, licorice plant with a slightly sweet and special odor; the active components in licorice such as triterpenoids, licorice flavonoids and licorice polysaccharides are natural and non-toxic and can improve animal performance when added to animal feed [25].R. Sajjadi [26] similarly showed that the addition of licorice to the diet has the potential to improve the energy status in the nutrition of dairy cows and increase the immune response of the animals. Moreover, licorice can improve its palatability when used as an herbal feed additive due to its slightly sweet odor.

Therefore, after studying and analyzing the above literature, we grasped the basic characteristics and relevance of chicken manure, straw, ruminants, and herbal drugs. Therefore, we set up the following experiments according to the way and ratios of feed industrial feed additives. It is used to avoid the problems related to the residue and resistance of antibiotics and chemical synthetic antimicrobial agents commonly used in feeding in animals and their secondary absorption in humans. Because according to Emily D. Richards et al. penicillin antibiotics used in bovine milk administration of β-lactam products may lead to very high concentrations of antibiotics in the udder of small ruminants, care must be taken to ensure that the food of small ruminants does not contain trace amounts of penicillin due to the possibility of allergic reactions to penicillin and penicillin derivatives in humans [27] . This misuse of antibiotics has started to threaten human health, while herbal additives of plant origin, being natural substances, avoid the drawbacks that other types of additives have. Therefore, the research on vigorously developing plant-derived herbal feed additives to replace exogenous antimicrobials has important economic and social benefits to solve the problem of antibiotic residues that have long plagued the development of animal husbandry, to develop a sustainable animal husbandry industry, and to meet people's needs for food safety.

3. Experimental design methods and parameter design ideas

Although the use of poultry manure as a feed additive for feeding ruminants has become widespread, the problem of misuse of antibiotics in feed additives is also rampant. Therefore, the development of a new sustainable feed additive for application in the cattle feeding industry to replace the use of antibiotics and other drugs and prevent diseases is the primary problem solving for the use of poultry manure for ruminant feeding today. Therefore, the purpose of this experiment is to design a new feed additive applied to cattle feeding technology based on a combination of herbs of plant origin (Baitou Weng Fang with dandelion, honeysuckle and licorice) with poultry manure (chicken manure) and plantation waste (corn straw) to improve the ecological pollution from poultry farming and agricultural waste and to ensure the sustainable development of human society and ecological environment. The optimal dosing ratio of herbs was fitted using experimental design parameters.

Experimental materials: chicken manure selected from caged laying hens, corn straw, molasses, and compound herbal substances. The combination of compound experimental herbs contains: Baidoung, Huangbai, Huanglian, Qinpi, Dandelion, Jinyinhua, Licorice.

Compound herbal medicine standard parameter dosage design: based on the weight of 80kg for young male, the dosage of herbal medicine are: Pulsatilla 10g , Huanglian 6g, Huangbai 8g,Qinpi 8g , Dandelion 8g, Jinyinhua 8g , Licorice 6g .Each kilogram of medicine per person is:Pulsatilla 0.125g, Huanglian 0.075g, Huangbai 0.1g , Qinpi 0.1g,Dandelion 0.1g,Jinyinhua 0.1g,Licorice 0.075g.Using the equivalence conversion method in fattening beef cattle, weighing approximately 250 kg, the drug weights for each 250 kg beef cattle were Pulsatilla 31.25g,Huang Lian 18.75g, Huangbai 25g,Qin Pi 25g,Dandelion 25g, Jinyinhua 25g and Licorice 18.75g.The herbs were crushed and powdered according to the corresponding proportions, and passed through a 40 mesh sieve. The pre-preparation process is shown in Figure 3-6.

Table 1. Compound herbal medicine standard parameter dosage design table

Pulsatilla (g)	Huang Lian(g)	Huang bai(g)	Qin Pi(g)	Dandelion(g)	Jinyinhua(g)	Licorice(g)	Total（g）
10	6	8	8	8	8	6	54
31.25	18.75	25	25	25	25	18.75	168.75


Figure 3. Pulsatilla


Figure 4. Huang Lian


Figure 5. Compound Medicinal Powder

Selection and grouping of experimental targets: Healthy and disease-free beef cattle (Aberdeen Angus) at the fattening stage with an average weight of about 250 kg were selected and randomly divided into 13 groups of 6 head each. On the basis of the feed formulation of MDK Jamee et al. The experiment was designed using the gradient experimental method with compound herbal medicine and corn straw as variables and the addition of chicken manure and molasses as quantitative. In the simulated gradient experiment, the T0 group was used as the control group, and the base amount of 168.75g of compound herbal medicine was added, and the gradient design of increasing and decreasing compound herbal medicine was carried out on the basis of T0. As follows: T0=30% cage excretion + 5% molasses + 65% corn straw + 0% compound herbal medicine; T1=30% cage excretion + 5% molasses + 64.5% corn straw + 0.5% compound herbal medicine; T2=30% cage excretion + 5% molasses + 64% corn straw + 1.0% compound herbal medicine; T3=30% cage excretion + 5% molasses + 63.5% corn straw + 1.5% compound herb; T4=30% cage excretion + 5% molasses + 63% corn straw + 2.0% compound herb; T5=30% cage excretion + 5% molasses + 62.5% corn straw + 2.5% compound herb; T6=30% cage excretion + 5% molasses + 62% corn straw + 3.0% compound herb T7=30% cage excretion + 5% molasses + 65.5% corn straw + (-0.5)% compound herbs; T8=30% cage excretion + 5% molasses + 66% corn straw + (-1.0)% compound herbs; T9=30% cage excretion + 5% molasses + 66.5% corn straw + (-1.5)% compound herbs; T10=30% cage excretion + 5% molasses + (-1.5)% compound herbs; T9=30% cage excretion + 5% molasses + 66.5% corn straw + (-1.5)% compound herbs cage excretion + 5% molasses + 67% corn straw + (-2.0)% compound herbal substance; T11=30% cage excretion + 5% molasses + 67.5% corn straw + (-2.5)% compound herbal substance; T12=30% cage excretion + 5% molasses + 68% corn straw + (-3.0)% compound herbal substance. The experiment was expected to last 30 days.

Experimental rations and feeding management：The supply of fine and coarse feeds, feeding methods, management measures and daily arrangements of the test cattle were carried out according to the conventional methods. The test cattle were grouped together in the same barn and fed twice a day, from 7:00 to 9:00 a.m. and from 19:00 to 21:00 p.m. Clean water was provided, and the barn was kept hygienic. The stable supply of various feeds was ensured during the experiment. In the article of Wang Jinhe et al. in the study of the effect of herbal additives on weight gain of beef cattle, it was shown that the daily weight gain was about 1.18 kg [28]. when applying compound herbal additives to beef cattle. For this experimental simulation design, the experimental beef cattle were weighed in the same order on an empty stomach at the beginning and end of the experiment, and each weighing was done for 2 consecutive days, and if the difference between the two weighings was more than 3%, the average weight was taken once more in the 3rd day, and finally the average daily weight gain was calculated, which was about 1.0 kg per beef cow, and then about 30 kg per 30 days, as shown in Table 2.

Table 2. experimental design parameter values

Chicken manure (%)	Chicken manure （Kg）	Molasses （Kg）	Corn straw(%）	Corn straw （Kg）	Compound Chinese Herbs （%）	Amount of compound herbs （g）	Estimated monthly weight gain（kg）
-3	5.25	0.875	68	11.9	-0.03	-5.0625	32.60
-2.5	5.25	0.875	67.5	11.812	-0.025	-4.21875	32.50
-2	5.25	0.875	67	11.725	-0.02	-3.375	32.35
-1.5	5.25	0.875	66.5	11.637	-0.015	-2.53125	32.17
-1	5.25	0.875	66	11.55	-0.01	-1.6875	32.19
-0.5	5.25	0.875	65.5	11.462	-0.005	-0.84375	32.52
0	5.25	0.875	65	11.375	0	0	32.85
0.5	5.25	0.875	64.5	11.2875	0.005	0.84375	32.86
1	5.25	0.875	64	11.2	0.01	1.6875	33.05
1.5	5.25	0.875	63.5	11.1125	0.015	2.53125	32.81
2	5.25	0.875	63	11.025	0.02	3.375	32.25
2.5	5.25	0.875	62.5	10.9375	0.025	4.21875	31.68
3	5.25	0.875	62	10.85	0.03	5.0625	31.24
Note: 0 is the design conversion standard value of 168.75g							

4.Dynamic linear simulation regression

The data were analyzed by using python software to determine the effect on the predicted monthly weight gain (kg) by decreasing or increasing the amount of straw and increasing or decreasing the number of herbs, while the content of chicken manure and molasses was kept constant. Based on the scatter plot, the scatter plot was fitted using polynomial, where the sample coefficient of determination of the three fits was the best and was 0.888, and the formula was y =-63564*x**3-893.73*x**2+29.435*x+18.71, and the analysis could be obtained that the predicted monthly weight gain was maximum when the herb content was at 1%. The linear regression plot is shown in Figure 7 below.

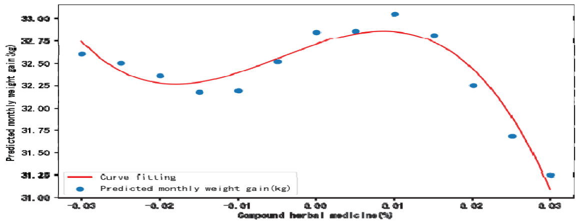

Figure 6. Linear regression plot

From the analysis of Figure 6 above, it can be obtained that the compound herb doping is the best doping ratio at both 1% and -1%, and the overall curve shows a linear regression. The regression scatter point is closest to the curve. Therefore, we conclude that the herbs added at 1% can best inhibit the bacteria in chicken manure, improve the gastrointestinal environment of beef cattle, and promote the growth and development of fattening cattle. This result is in general agreement with the experimental results of Wang Xiaowen's study on the growth performance of beef cattle using different additions of several herbal formulations in combination with yellow corn straw silage [29]. Thus, our simulation results can be proved to be feasible.

5. Conclusion

Thus, the purpose of this paper was to demonstrate the feasibility of compound herbs as feed additives for cattle feeding technology and to reduce the pollution of the habitat environment through the consumption of poultry manure and agricultural straw waste. The plant herbal properties of herbs without side effects are used to replace antibiotic abuse in ruminants as a way to ensure human food security issues.

This study aims to predict the optimal experimental dosing of regression herbs through linear simulation analysis in order to provide accurate range data for later extensive experiments. The experimental time and money costs are reduced to promote maximum economic value benefits.

In conclusion, our simulation data showed that the compound herbs can be effectively used as feed additives for ruminant feeding at 1%. Thus, it also verifies the feasibility of compound herbal substances as feed additives for cattle feeding technology, so that the application of antibiotics can be replaced in livestock farming compound herbal substances to avoid problems such as antibiotic residues, thus better promoting the sustainable development of livestock farming, improving the ecological environment and human health, and can lead to the green development of the economy.

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