

Effects of Dietary Supplementation with a Combined Probiotic of Lactic Acid Bacteria and Yeast during Late Gestation on Sow Reproductive Performance, Plasma Lipid Metabolism, and Antioxidant Capacity (Postprint)

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Abstract

This experiment was conducted to investigate the effects of dietary supplementation with a compound bacteria preparation of lactic acid bacteria and yeast during late gestation on sow reproductive performance, plasma lipid metabolism, and antioxidant capacity, and to determine the optimal supplementation level. Forty-eight third-parity “Large × Landrace” hybrid sows with similar body condition at day 80 of gestation were randomly allocated to 4 groups with 12 replicates per group (1 sow per replicate). The control group was fed a basal diet, while groups I, II, and III were fed the basal diet supplemented with 150, 300, and 450 mL/d of lactic acid bacteria and yeast compound bacteria fermentation broth, respectively. The pre-trial period lasted 5 days, and the formal trial period lasted 37 days. The formal experiment commenced at day 85 of gestation and concluded at day 7 postpartum, with no supplementation of the compound bacteria fermentation broth during the 7-day postpartum period. The results showed that, compared with the control group: 1) litter birth weight was significantly increased in group II ($P < 0.05$), and piglet birth weight was significantly increased in groups I, II, and III ($P < 0.05$); 2) plasma total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-C) contents were significantly increased in groups I and II ($P < 0.05$), and plasma triglyceride (TG) content was significantly increased in group II ($P < 0.05$); 3) plasma malondialdehyde (MDA) content was significantly decreased in groups I and III ($P < 0.05$). It was concluded that under the experimental conditions, the optimal supplementation level of the compound bacteria fermentation broth was 300 mL/d based on reproductive performance and plasma lipid metabolism, whereas 150 mL/d was optimal based on plasma antioxidant capacity and economic benefits.

Full Text

Effects of Dietary Compound Bacteria of Lactobacillus and Yeast in Late Pregnancy on Reproductive Performance, Plasma Lipid Metabolism and Antioxidant Capacity of Sows

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Abstract

This experiment was conducted to investigate the effects of dietary compound bacteria of Lactobacillus and yeast in late pregnancy on reproductive performance, plasma lipid metabolism and antioxidant capacity of sows, and to determine the optimum supplementation level of compound bacteria in diets of late pregnancy sows. Forty-eight 3-parity “Large White × Landrace” hybrid sows with similar body condition at day 80 of gestation were randomly divided into 4 groups with 12 replicates per group and 1 sow per replicate. Sows in the control group were fed a basal diet, while those in groups , and were fed the basal diet supplemented with 150, 300 and 450 mL/d of compound bacteria fermentation broth of Lactobacillus and yeast, respectively. The pre-trial period lasted for 5 days, and the formal trial period lasted for 37 days from day 85 of gestation to day 7 postpartum, during which no compound bacteria fermentation broth was added to the diets in the first 7 days postpartum. The results showed that, compared with the control group: 1) the birth litter weight of piglets in group was significantly increased ($P < 0.05$), and the birth weight of piglets in groups , and was significantly increased ($P < 0.05$); 2) the plasma contents of total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-C) in farrowing sows in groups and were significantly increased ($P < 0.05$), and the plasma triglyceride (TG) content in group was significantly increased ($P < 0.05$); 3) the plasma malondialdehyde (MDA) content in farrowing sows in groups and was significantly decreased ($P < 0.05$). It was concluded that, under the conditions of this experiment, the optimum supplementation level of compound bacteria fermentation broth was 300 mL/d for reproductive performance and plasma lipid metabolism, and 150 mL/d for plasma antioxidant capacity and economic benefits.

Key words: compound bacteria of Lactobacillus and yeast; pregnant sows; reproductive performance; plasma lipid metabolism; plasma antioxidant capacity

Probiotics as alternatives to antibiotics have become a major research focus in pig production, with broad effects and good application results. Lactobacillus is a type of bacteria that can utilize carbon sources to produce lactic acid [1], which can enhance animal immunity, improve gastrointestinal flora balance, provide

antioxidant effects, exhibit anti-tumor and anti-hypertensive properties, and reduce cholesterol levels [2]. Yeast is a widely used probiotic that can enhance animal immunity, improve gastrointestinal flora balance, regulate metabolism, and provide nutritional sources [3]. Lactobacillus and yeast are two beneficial probiotics for sows that can coexist in the same system. Research has found that the metabolites of Lactobacillus can provide carbon sources for yeast [4], while the nutritional factors produced by yeast fermentation can be utilized by Lactobacillus [5], and the metabolites of the two can be mutually utilized [6]. Probiotics can improve sow reproductive performance, enhance immunity, and improve the growth performance of offspring piglets. Li et al. [7] found that adding a compound bacteria of Lactobacillus, yeast and Bacillus subtilis to diets could improve the reproductive performance of lean-type sows. Yan et al. [8] found that adding a compound preparation of phytase, complex enzymes, Bacillus subtilis, yeast and Lactobacillus could improve the production performance of pigs in the late fattening stage on fermentation beds. Late pregnancy is the fastest period of fetal growth and development, with rapid fetal weight gain. The nutritional level of sows directly affects fetal growth, piglet birth litter weight and individual weight, and lactation performance during the nursing period. Adding probiotics to sow diets in late pregnancy can improve gastrointestinal flora balance, increase feed utilization efficiency, enhance sow immunity, and reduce disease incidence, though results vary due to different experimental factors. This experiment aimed to study the effects of adding different doses of compound bacteria of Lactobacillus and yeast to sow diets in late pregnancy on reproductive performance, plasma lipid metabolism and antioxidant capacity, determine the optimal dosage of compound bacteria, and provide a scientific basis for the rational application of probiotics in sow production.

1.1 Experimental Materials

The compound bacteria preparation, including Lactobacillus, yeast and their shared compound culture medium, was purchased from Shenzhen Bai' aofei Biological Company. The product was milk-yellow powder with yogurt aroma. After fermentation in a fermentation tank for 24 hours, it was fed to sows in the form of biological fermentation broth. The fermentation broth had a pH of 3.59, containing Lactobacillus at 1.0×10^8 CFU/mL and yeast at 8×10^8 CFU/mL.

1.2 Experimental Design

The feeding trial was conducted from February to April 2017 at Tianzhong Pig Farm in Luyi County, Henan Province. Forty-eight 3-parity "Large White \times Landrace" hybrid sows with similar body condition at day 80 of gestation were randomly divided into 4 groups with 12 replicates per group and 1 sow per replicate. The control group was fed a basal diet, while groups , and were fed the basal diet supplemented with 150, 300 and 450 mL/d of compound bacteria fermentation broth of Lactobacillus and yeast, respectively. The pre-trial period lasted for 5 days, and the formal trial period lasted for 37 days from

day 85 of gestation to day 7 postpartum, during which no compound bacteria fermentation broth was added to the diets in the first 7 days postpartum. The basal diet was formulated according to NRC (2012) nutrient requirements for sows, and its composition and nutrient levels are shown in Table 1 .

1.3 Feeding Management

Pregnant sows were managed according to conventional farm procedures. Late pregnancy sows were housed in large pens and transferred to farrowing crates 3 days before the expected delivery date for individual pen housing. Control and experimental group sows were housed in the same gestation barn and farrowing rooms. Both late pregnancy and the first 7 days of lactation involved restricted feeding, with late pregnancy sows fed 1.8 kg per head per meal twice daily. No feed was provided on the day of delivery, 0.5 kg per head per day on day 1 postpartum, increasing by 0.5 kg daily thereafter, and ad libitum feeding after 7 days. Diets for all groups were mixed with appropriate water and fed as wet mash, with groups , and receiving different doses of compound bacteria fermentation broth. Sows had unlimited access to water and were housed in the same environment with routine immunization according to farm procedures.

1.4.1 Reproductive Performance

Reproductive performance indicators for each experimental sow were recorded, including total litter size, live litter size, mummy piglets, stillbirths, and piglet birth weight. The live litter rate, mummy rate, stillbirth rate and piglet birth litter weight were calculated.

1.4.2 Plasma Lipid Metabolism and Antioxidant Indices

On day 85 of gestation (fasting) and on the day of delivery, 10 mL of fresh blood was collected from the ear vein of each sow using heparin sodium anticoagulant vacuum tubes. After standing for 30 minutes, the blood was centrifuged at 3,000 r/min for 20 minutes, and the plasma supernatant was collected and stored at -20°C. Plasma lipid metabolism indices included total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) contents, which were measured using an automatic biochemical analyzer. Plasma antioxidant indices included malondialdehyde (MDA) content and superoxide dismutase (SOD) activity, which were measured using a colorimetric method with kits purchased from Beijing Huaying Biotechnology Research Institute.

1.5 Data Processing

Experimental data were preliminarily processed using Excel 2007 software and then subjected to one-way ANOVA using SPSS 19.0. Significant differences were tested using Duncan' s multiple comparison method. Results were expressed as "mean \pm standard deviation" , with $P < 0.05$ indicating significant difference.

2.1 Effects of Compound Bacteria of Lactobacillus and Yeast on Sow Reproductive Performance

As shown in Table 2 , there were no significant differences among groups in total litter size, live litter size, mummy piglets, stillbirths, live litter rate, mummy rate or stillbirth rate ($P>0.05$). Compared with the control group, the birth litter weight of piglets in group was significantly increased ($P<0.05$), while groups and showed no significant difference ($P>0.05$). The birth weight of piglets in groups , and was significantly increased ($P<0.05$).

2.2 Effects of Compound Bacteria of Lactobacillus and Yeast on Sow Plasma Lipid Metabolism Indices

As shown in Table 3 , there were no significant differences in plasma TC, TG, HDL-C and LDL-C contents among groups on day 85 of gestation ($P>0.05$). As shown in Table 4 , there was no significant difference in plasma LDL-C content among groups in farrowing sows ($P>0.05$). Compared with the control group, plasma TC content in groups and was significantly increased ($P<0.05$), while group showed no significant difference ($P>0.05$). Plasma TG content in group was significantly increased ($P<0.05$), while groups and showed no significant difference ($P>0.05$). Plasma HDL-C content in groups and was significantly increased ($P<0.05$), while group showed no significant difference ($P>0.05$).

2.3 Effects of Compound Bacteria of Lactobacillus and Yeast on Sow Plasma Antioxidant Indices

As shown in Table 5 , there were no significant differences in plasma MDA content or SOD activity among groups on day 85 of gestation ($P>0.05$). Compared with the control group, plasma MDA content in farrowing sows in groups and was significantly decreased ($P<0.05$), while group showed no significant difference ($P>0.05$). There were no significant differences in plasma SOD activity among groups in farrowing sows ($P>0.05$).

3.1 Effects of Compound Bacteria of Lactobacillus and Yeast on Sow Reproductive Performance

The reproductive performance of sows in late pregnancy is mainly manifested in litter size of live piglets, number of stillbirths, birth litter weight of piglets, individual birth weight of piglets, live litter rate and stillbirth rate. Sow litter size is related to sow breed and nutritional level in early pregnancy [9]. Sow nutrition and health directly affect their reproductive performance and the growth and development of offspring piglets [10]. Adding probiotics to sow diets in late pregnancy can enhance sow constitution and improve feed utilization efficiency, thereby improving reproductive performance. However, research results on the effects of probiotics on reproductive performance of pregnant sows vary, possibly due to differences in sow breed, pregnancy stage, feeding environment, feeding management, nutritional level, and probiotic species, quantity and ratio.

Liu et al. [11] found that adding compound probiotics of *Bacillus*, *Lactobacillus* and yeast to sow diets 30 days before delivery could significantly increase individual birth weight of piglets, but had no significant effect on litter size. Huo et al. [12] found that adding microecological preparations to sow diets 45 days before delivery could significantly increase litter size, live litter size and piglet birth weight, and significantly reduce stillbirth rate. Fu et al. [13] found that adding compound bacteria preparations to purebred Landrace multiparous sow diets 45 days before delivery could significantly increase piglet birth litter weight and piglet birth weight, but had no significant effect on number of newborn piglets, live newborn piglets or survival rate of newborn piglets. This study found that adding compound bacteria of *Lactobacillus* and yeast to sow diets in late pregnancy could significantly increase piglet birth litter weight and piglet birth weight, possibly because the compound bacteria preparation enhanced sow absorption and digestion of nutrients in the diet and optimized the intestinal environment. This study also found that compound bacteria had no significant effect on reproductive performance indicators such as litter size, live litter size, mummy piglets, stillbirths, live litter rate, mummy rate and stillbirth rate in late pregnancy sows, which is consistent with the research results of Zhang et al. [14] and Long [15].

3.2 Effects of Compound Bacteria of *Lactobacillus* and Yeast on Sow Plasma Lipid Metabolism

TC is an essential nutrient for animals [16], an important component of cell structure, and a raw material for the synthesis of steroid hormones such as sex hormones, vitamin D3 and bile acids [17]. It can maintain the physiological function of white blood cells and the integrity of blood vessel walls, reduce cancer rates, and promote immune responses by initiating T cell production of the immune molecule interleukin-2 (IL-2), inducing T lymphocyte proliferation and promoting B lymphocyte immune responses [18]. TC is often transported in blood through high-density lipoprotein (HDL) and low-density lipoprotein (LDL) [19]. Excessive LDL-C content indicates excessive TC in blood circulation and high blood lipid content, and studies have shown that increased LDL-C content is a danger signal for arteriosclerosis [20]. HDL-C can remove excess LDL-C from blood vessel walls, protect blood vessels and reduce the incidence of arteriosclerosis [21]. Increased blood lipid content within a certain range is beneficial for pregnant mothers and fetuses [22]. Fat metabolism is enhanced in late pregnancy, with increased free fatty acids and glycerol content in maternal plasma that can serve as priority effective substances for maternal glucose synthesis and energy storage. When maternal nutrition is deficient in late pregnancy, this ensures continuous input of required nutrients to the fetus while the mother can utilize its own fat metabolites to maintain metabolic balance [23]. However, excessively high blood lipid content and abnormal lipid metabolism can lead to placental abruption and pregnancy-induced hypertension syndrome [22]. Probiotics have lipid-lowering effects [24], and studies have found that *Lactobacillus* can reduce blood TC content. The mechanism of TC degradation

by *Lactobacillus* is not yet clear and is still under investigation, with several main theories: 1) absorption theory - *Lactobacillus* degrades TC by absorbing it from the medium, with different bile salt concentrations affecting the absorption rate [25-27]; 2) precipitation theory - studies have found that bile salt hydrolase produced by *Lactobacillus* is a key factor in reducing TC content, as it can change bile salt structure, causing bile salts to form precipitates with TC that are excreted from the body, thereby reducing TC content [28-29]; 3) incorporation into cell membrane theory - studies have found that bacteria absorb TC, but not all absorbed TC enters the cell interior, with some entering the cell membrane, and there are other degradation mechanisms on the cell membrane surface [30-31]. The results of this study showed that, compared with the control group, plasma TC, TG and HDL-C contents in group farrowing sows were significantly increased, but there was no significant difference in plasma LDL-C content among groups. This indicates that compound bacteria cannot reduce blood lipid content in farrowing sows, but can promote lipid metabolism and protect the integrity of arterial blood vessels. Studies have shown that higher serum TG content in pregnant women is associated with greater fetal birth weight, though other studies have shown no correlation between fetal birth weight and maternal blood lipid content, and there is currently no unified conclusion [22]. This study found that piglet birth litter weight was in the order group > group > group > control group, and plasma TG content in farrowing sows followed the same pattern, consistent with the result that higher serum TG content is associated with greater fetal weight.

3.3 Effects of Compound Bacteria of *Lactobacillus* and Yeast on Sow Plasma Antioxidant Capacity

Oxidative stress is an imbalance between the antioxidant defense system and oxide generation when the body's ability to generate oxides exceeds the antioxidant defense system's ability to clear them or when the antioxidant defense system's capacity is weakened, and is also an imbalance between generation and degradation of reactive oxygen species [32-33]. Appropriate reactive oxygen species content is necessary for normal physiological activities, but excessive reactive oxygen species can damage normal physiological functions. The main indicators that reflect oxidative damage and antioxidant defense are MDA and SOD [34]. MDA is a degradation product of lipid peroxides formed by the combination of plasma lipids and free radicals, and can serve as an indirect indicator of free radical content in the body. Reduced plasma MDA content indicates decreased free radical content and increased defense capacity of the antioxidant system, reflecting enhanced antioxidant capacity. SOD is one of the main endogenous antioxidant enzymes that scavenge free radicals in animals. Increased SOD activity indicates enhanced antioxidant defense system capacity and antioxidant capacity, while decreased activity indicates weakened antioxidant capacity [34]. Studies have proven that *Lactobacillus* has antioxidant activity [35], with antioxidant substances mainly including antioxidant enzymes, manganese ions and thiol compounds [36], though the specific antioxidant mechanism of

Lactobacillus is not yet fully clear and may include several pathways: 1) antioxidant enzyme mechanism - the main antioxidant enzymes in Lactobacillus include SOD, catalase (H₂O₂), and nicotinamide adenine dinucleotide (NADH) oxidase/NADH peroxidase; 2) manganese ion antioxidant mechanism - studies have found that high concentrations of manganese ions in Lactobacillus have antioxidant effects similar to SOD [37]; 3) thiol compound antioxidant mechanism - Lactobacillus has protein thiol and non-protein thiol compounds that can scavenge hydroxyl radicals and exert antioxidant effects [36]; 4) adaptive mechanism - Lactobacillus under certain environmental stresses can enhance defense capabilities against stress responses, such as producing large amounts of oxygen free radicals under certain conditions that induce antioxidant responses in Lactobacillus [37]. This study found that adding compound bacteria of Lactobacillus and yeast to sow diets in late pregnancy had no significant effect on plasma SOD activity in farrowing sows, but plasma MDA content in groups and was significantly lower than in the control group. This indicates that compound bacteria can reduce free radical content, decrease oxide generation, and enhance antioxidant defense system capacity.

4 Conclusion

Adding compound bacteria of Lactobacillus and yeast to sow diets in late pregnancy can improve sow reproductive performance, plasma lipid metabolism and antioxidant capacity, with significant effects on piglet birth litter weight and individual birth weight, as well as plasma TC, TG, HDL-C and MDA contents in farrowing sows. Under the conditions of this experiment, the optimum supplementation level of compound bacteria fermentation broth was 300 mL/d for reproductive performance and plasma lipid metabolism, and 150 mL/d for antioxidant capacity and economic benefits.

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