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Case report

The effects of *Ganoderma lucidum* compound on goat weight and anti-inflammatory: a case study of circular agriculture

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Abstract: Green sustainable development is an extremely important concept, and the most representative industry is agriculture. The agricultural industry value chain covers the connotation of green and sustainable innovation. Circular agriculture is a revival of old agricultural practice, and the concept is a combination of crop planting and livestock farming to minimize the losses in the food production chain. This study explores the effects of Ganoderma lucidum compound added in feed on goat weight and anti-inflammatory through a case study. This study uses average weight difference analysis and an independent t-test to verify the goat weight gain and growth, and uses nitric oxide, interleukin 6, tumor necrosis factor α, and tetrazolium to verify goat health. This study shows that dietary supplementation of the Ganoderma lucidum compound has better performance in weight gain and growth of goats. It also provides a method of reducing antibiotics to promote health and welfare in the goat or even the livestock breeding industry. At the same time, it may help livestock owners to improve management efficiency.

Keywords: circular agriculture; food production chain; crop planting; livestock farming; Ganoderma lucidum compound

1. Introduction

Closed loop supply chains (CLSCs) have attracted growing attention over the last two decades, from both academia and practitioners, owing to their increasing economic importance and tightening environmental legislation [1,2]. CLSCs can create enormous economic potential by recycling products and recovering added value [3]. CLSCs are to take back goods from customer and recovering added value through reusing the entire goods. That is, CLSCs manage a system to maximize added value over entire life cycle of goods with value recovery from returns [2]. A recoverable goods environment is a CLSC with both traditional logistics forward flows and reverse logistics channels [4,5]. The concept of recycling originated from industrial ecology, aiming to reduce resource consumption and the burden on the environment through a closed material cycle. Under this range, the goal is not only to prevent the loss of materials and substances, but also to recycle them [6,7]. In accordance with this principle, this study aims to develop a circular food system, with the aim to minimize the input of resources and use practical technologies. The proposed system can encourage the use of renewable resources to prevent the consumption of natural resources, and maximize the value of the food system in order to reduce the decline of resources.

In order to solve the extreme climate and deteriorating environmental problems, many environmental protection organizations and people have put forward different solutions. Green sustainable development is an extremely important concept, and the most representative industry is agriculture. Due to the disruption of the global agri-food supply chain, the COVID-19 pandemic has enormously exacerbated food security issues [8,9]. The agri-food supply chain should give thought to reducing food waste and developing ecosystem circulation in all its links in order to strengthen adaptability [8,10,11]. The agri-food supply chain covers the connotation of green and sustainable innovation from front-end creative research and development to intermediate design, processing and manufacturing, and even back-end marketing services. Therefore, the key issue is the innovation of the value of products and services through an excellent agricultural industry value chain while taking into account the sustainable development of the economy and the environment so that agriculture can obtain a lasting advantage in the current technological competition environment. Circular agriculture is the revival of practices in the old agricultural era. This concept is to combine crop planting and animal husbandry in order to minimize losses in the food production chain. Before the 1900s, this idea of mixed farming had always been the dominant player in agriculture. For example, pigs, cows, goats, and chickens eat the by-products of crops, or the scraps of agricultural materials, or else eat leftovers from human beings, and then return their excrement and feces to the land. An alternative to specialized agriculture is to integrate crop planting and livestock production within the farm. The integrated agriculture of planting crops and raising livestock can improve soil quality, increase yield, produce a variety of foods, increase pollinators, help prevent pests and diseases, and improve land use efficiency [12,13]. Wezel et al. [14] defined agro-ecological areas as sites for sustainable agriculture and transitional food systems. At the same time, three main areas must be considered, namely suitable agricultural practices, protection of biodiversity and natural resources, and embedded food system development. These three areas are also an indispensable part of the

agricultural ecology field.

In this study, through this mixed farming model, the scraps of *Ganoderma lucidum* compound (including *Ganoderma lucidum*, Chinese wolfberry, gardenia, and soybeans), which were originally discarded, were added to the feed to improve the fattening and growth traits of goats. These scraps all come from the product characteristics such as appearance defects, poor sales, and immature harvesting. In addition, the excrement of the goat tested in this study is piled up for a period of time, and then sent to the crop growers to make compost for planting crops. For this reason, this study uses a mixed farming model to practice and apply the circular economy of waste recycling to meet the goals of circular agriculture. Based on this concept, this research thoroughly explores the effects of Chinese herbal medicine: *Ganoderma lucidum* compound on the fattening and growth traits of goats, and makes full use of the application of circular agriculture. Moreover, studies have shown that water extract of gardenia reduces the production of nitric oxide (NO), interleukin 1β (IL-1β), interleukin 6 (IL-6), ROS and prostaglandin E2 (PGE2) induced by lipopolysaccharide (LPS) in bathophenanthrolinedisulfonate 2 (BPS-2) cells [15].

Mutton is one of the main products of goat breeding. Breeding goats, young rams, and ewes that have lost reproductive capacity are often sold by their owners for slaughter after fattening. The fattening of goats can increase meat volume, improve meat quality, and/or produce good quality fur in the short term. Under the fattening principle, it is necessary to increase the storage of nutrients in goats, while reducing their consumption of nutrients. Successful fattening of goats can increase the operating efficiency of the entire ranch; thus, it is necessary to adopt different feeding methods, management models, and nutritional requirements, in order to meet the growth of goats at different stages.

However, these growth needs, in addition to the feeding methods and nutritional requirements need to take into consideration of birth season, gender and genetics. Studies have explored the correlations between Creole goat breeds or hybrids and seasons, using seasonal practical management, in order to improve the birth weight and growth rate of young goats. The results have confirmed that the season has a significant impact on birth weight [16]. There are various factors that affect the birth weight of Nubian goats in Sudan, such as gender. The weight of males is heavier than that of females [17]. The birth weight of a male lamb born in the Sirohi breed is higher than that of a female lamb born in single, twins and triplets [18]. Another study pointed out that under the condition of ad libitum intake and supplementation, the weight gain of small East African goats is significantly improved, and a cost-benefit analysis is recommended [19].

The addition of refined feed to the daily grain can significantly enhance the weight gain effect, indicating that the fattening effect of goats is highly correlated with the feeding mode during the fattening period [20]. The reproduction of Sudanese desert goats born in autumn is heavier than those born in winter and summer, which may be attributed to better pastures and good weather in autumn as well as the supplement of indoor nutrients [21].

In recent years, research on goats has been carried out in a different way. For example, the addition of green tea by-products as feed additives showed a positive effect on growth performance, meat quality, blood metabolites, and immune cell proliferation [22]. The addition of polyphenols in goat diets can prevent the oxidative deterioration of lipids and proteins and improve the meat quality of livestock [23]. Legumes (Indigofera zollingeriana) have the characteristics of concentrated feed,

which can increase daily body weight and feed utilization efficiency as the proportion increases [24].

Based on the above research, livestock owners can improve management efficiency by adding specific refined feed to livestock feed during the fattening period, which is the main axis of this study.

2. Literature review

The use of antibiotics in the animal husbandry industry worldwide is estimated to be almost twice that of humans. However, the use of such antibiotics in the agricultural and animal husbandry businesses of many countries is not simply for treating diseases or preventing infections, but to accelerate animals' growth, which is not a sustainable model [25]. Therefore, in order to avoid this crisis, the European Union began to restrict the use of antibacterial agents in 1997 and banned the use of avoparcin to promote animals' growth, and in 2006, banned the remaining growth promoters [26].

In recent years, some scientific evidences have successively proved the contribution of Chinese herbal medicine to poultry breeding. In addition to providing human applications, *Ganoderma lucidum* can also be used in the livestock industry under proper cost control. In addition to improving the disease resistance and immunity of poultry and livestock, it also can reduce the use of antibiotics and other animal drugs. Research has indicated that *Ganoderma lucidum* has been widely used in Eastern countries for more than two thousand years [27], and its efficacy has been extensively studied and scientifically verified. There are more than 400 different biologically active compounds in the ingredients. The main ingredients include triterpenoids, polysaccharides, nucleotides, sterols, proteins and trace elements [28]. At a concentration of 12.5μM, *Ganoderma lucidum* has an inhibitory effect on the inflammation caused by nitric oxide (NO), and the inhibition rate is 45.5% [29]. When being used to treat inflammatory diseases, *Ganoderma lucidum* triterpenes can inhibit NF-κB and MAPK induced by TLR4-MyD88 [30]. Ganoderma's anti-oxidation and free radical scavenging effects on different animal models *in vivo* and *in vitro* can produce immune regulation, anti-tumor, antihypertension, hypoglycemic, brain, liver, cardiovascular, kidney, and anti-aging effects, along with other pharmacological effects [31]. The consumption of *Ganoderma lucidum* polysaccharides can alleviate colitis induced by dextran sodium sulfate, and reduce ulcerative colitis [32]. The mycelium derived from *Ganoderma lucidum* has the effect of regulating intestinal function [33]. This study aims to explore the effect of *Ganoderma lucidum* compound added in feed on the weight gain of goats.

Although more intensive feeding methods have been adopted for general livestock, due to the changes of external environments and the threat of multiple pathogens, antibiotics or growth promoters are unavoidably used to improve livestock performance or prevent some diseases. However, long-term or improper use of antibiotics may easily lead to the development of drug-resistant strains, the risk of drug residues in animal products, the imbalance of microorganisms in soil and water, or even the outcome of ecological disturbance and environmental pollution [34]. Similar problems have occurred in countries around the world. Therefore, the European Union began to ban the use of all non-therapeutic antibiotics in 1996 [35] in order to avoid drug residues and environmental damage. Studies have also pointed out that the widespread use of non-therapeutic

antibiotics in animals has resulted in human resistance to antibiotics [36].

In summary, adding *Ganoderma lucidum* to livestock feed could promote growth, and regulate immunity, intestinal pathology and inflammation; however, whether it has an effect on the fattening and growth of goats has not yet been discussed in related literature reports. Therefore, this study explores the addition of *Ganoderma lucidum* compound in feed for the fattening and weight gain of goats.

3. Research methods

3.1. Experimental design

Most Taiwanese goats are raised in small and medium-sized pastures. This research site is located in the Anglo-Nubian (*Capra hircus*) goat pasture in Miaoli County, Taiwan. The rancher in the pasture has more than 30 years of professional experience to cooperate with the experimental work of this study. This study performed segmentation and selected suitable goats, with the assistance of professional veterinarians, including selecting male goats born in the same month with the same or different fetuses. A four-month field trial was conducted from August 13, 2016 to December 12, 2016. Nevertheless, the pasture in this study is a small professional goat farm which can only provide 12 growing male goats with a weight of about 40 to 50kg suitable for the weight gain test. The male goats were randomly divided into a control group and a test group, with six goats in each group. This study was divided into two stages. The first three months (August 13, 2016 to November 12, 2016) was the first stage, during which *Ganoderma lucidum* compound was not added to the feed for both groups. All goats were fed according to the general method. The feed was mainly corn and soybean meal until the weights of the goats reached about 50 to 55kg. The fourth month (November 13, 2016 to December 12, 2016) was the second stage, during which 20 grams of *Ganoderma lucidum* compound was added to the feed of the test group daily, and no *Ganoderma lucidum* compound was added to the feed of the control group. The average weight difference analysis and independent t-test were conducted using the SPSS software to find out whether adding *Ganoderma lucidum* compound could help goats gain weight.

3.2. Screening methods for inflammatory factors

In this study, the mouse macrophage cell line (Raw 264.7) was used as the experimental model to explore the *Ganoderma lucidum* compound's anti-inflammatory activity. This study used the lipopolysaccharide (LPS) of the Gram-negative bacterial cell wall as the antigen, which has assisted in stimulating the cells to produce inflammatory reactions and promote the generation of inflammatory mediators. Simultaneously, the *Ganoderma lucidum* compound was added to analyze the production change of inflammatory mediates, and to evaluate the application potential of *Ganoderma lucidum* compound in reducing inflammation. These items, which have been analyzed, included three inflammation-related factors: NO, IL-6, and tumor necrosis factor α (TNF-α). In addition to the analysis of inflammatory factors, cell viability analysis: tetrazolium (MTT) assay was also performed to monitor whether the added extract is toxic to macrophages. This study used NO,

IL-6, TNF-α, and MTT to verify goat health.

3.2.1. Sample of Ganoderma lucidum compound

The *Ganoderma lucidum* compound was first dried to 60 ℃ for 8–10 hours, and then grinded and sieved within the grinder. The particle size was weighted at 60 meshes (250 microns), and the moisture content of the *Ganoderma lucidum* compound was at 5% or lower. For the sample treatment, over 100mg of *Ganoderma lucidum* compound powder was added with 10ml of pure water, and shook at 150 rpm for 2 h at 25 ℃ before it was centrifuged at 5000 rpm for a precise period of 5 min. The upper aqueous solution was collected as the finalized procedure.

3.2.2. Cell culture, inflammatory analysis, and MTT analysis

The cell RAW 264.7 (BCRC 60001) used in the experiment was purchased from Food Industry Development Institute, Taiwan. For the production of macrophages of BALB/c mice, the cell culture was carried out within Dulbecco's Modified minimal Essential Medium (DMEM) containing 100I.U./ml penicillin and 100μg/ml streptomycin, yet consisting of 10% fetal bovine serum (FBS).

First, the cells were placed in a constant temperature cell incubator (37 ℃, 5% CO_2). When the cells grew from 7 to 8 cm, they were subcultured. The method for measuring nitric oxide (NO) produced by cells was to inoculate 1×10^5 cells per well in a 96-well plastic cell culture dish, place it in a constant temperature cell incubator for 24 h, and then add different concentrations of *Ganoderma lucidum* compound and LPS (10 μg/ml), and analyze after 24 h of incubation.

3.2.2.1. NO analysis

Next, 100μl/well of culture medium was taken and added to an equal amount of freshly prepared Griess reagent [from Griess A reagent (0.1% N-(1-Naphthtl) ethylenediamine) and Griess B reagent (1% Sulfanilamide / 5% H_3PO_4) mixed in equal proportions]. Then, after sitting for 5 min in the darkness, the absorbance at 540nm (OD 540) was measured with an enzyme immunoassay analyzer. The result was a sample with a known concentration of sodium nitrite ($NaNO_2$) as the standard of this experiment being made. The interpolation method was used to calculate the concentration of nitrite in the sample as well.

3.2.2.2. Analyses of IL-6 and TNF-α

Mouse IL-6 ELISA Max and Mouse TNF-α ELISA Max analysis kits were purchased from Biolegend, which were being used earlier. Each enzyme-linked immunosorbent assay (ELISA) was performed by using a double-antibody sandwich binding method. The second-stage antibody was conjugated with horseradish peroxidase (HRP) enzyme, colored with 3,3',5,5'-tetramethylbenzidine (TMB), and then it would be ready to be used to measure by absorbance at 450nm using an ELISA reader. The concentrations of IL-6 and TNF-α in the samples were converted after being compared with the reference standard's concentration.

3.2.2.3. MTT cell survival rate analysis

The RAW264.7 mouse macrophage cell line was inoculated in a 96-well plate culture at a cell density of 1×10^5 cells/well. After the cells were being attached, the test sample and LPS were added. This treatment lasted for 24 h, and 20μl of freshly prepared MTT (3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) were added to each well. After that, the sample was returned to the cell culture incubator for more than 4 h. Then, after removing the supernatant, 50μl of dimethyl hydrazine (DMSO) was added, and the mixture was shaken for over 10 min. Finally, a disc enzyme analyzer was used to measure the light absorption value at a wavelength of 540nm.

4. Research Results

4.1. Average weight difference analysis results

This study used the difference analysis of average weight and independent t-test to compare the weight difference between the control group and the test group, and also to confirm whether the weight gain of the test group is better than that of the control group.

The basic statistical results of the control group and the test group are shown in Table 1. A four-month field trial was conducted from August 13, 2016 to December 12, 2016. A total of 12 goats were reared in the trial, and two groups were randomly divided. All 12 goats were weighed before the test. In the first three months (August 13, 2016 to November 12, 2016), no *Ganoderma lucidum* compound was added to the feed for both groups, and in the fourth month (November 13, 2016 to December 12, 2016), the *Ganoderma lucidum* compound was added to the feed for the test group.

Table 1. Basic statistics of goat weight gain for control group and test group.

Date	Control/test group	Quantity	Maximum weight gain (kg)	Minimum weight gain (kg)	Average weight gain (kg)	Standard deviation of weight gain (kg)
2016/08/13~09/12	control	6	7.20	3.55	5.425	1.230
	test	6	7.80	3.45	4.858	1.595
2016/09/13~10/12	control	6	6.20	2.90	4.633	1.319
	test	6	7.90	2.60	5.200	1.929
2016/10/13~11/12	control	6	7.80	0.70	4.717	2.519
	test	6	6.60	0.30	3.183	2.169
2016/11/13~12/12	control	6	7.70	1.00	4.017	2.252
	test	6	9.50	5.50	7.683	1.703

Table 1 lists basic descriptive statistics of goat weight gain for the control group and the test group. According to the test results on December 12, 2016, there was no difference in weight gain between the control group and the test group in the first three months. However, when the *Ganoderma lucidum* compound was added to the test group starting the fourth month, the highest

gain of the test group was 9.5kg, the lowest gain was 5.5kg, and the average gain was 7.683kg, which was better than the control group (the highest gain was 7.7kg and the lowest was 1.0kg, the average was 4.017kg). The average weight gain of the test group was higher than that of the control group (3.666kg). In addition, in terms of standard deviation, the standard deviation of the test group was 1.703, which was more stable than the 2.252 of the control group.

Table 2 lists the analysis results of average weight gain for the control group and the test group. A four-month field trial was conducted from August 13, 2016 to December 12, 2016. According to the statistics of the results on December 12, 2016, there was no difference in average weight gain between the control group and the test group in the first three months. However, since the fourth month, the test group's average weight gain was 14.04%, which was better than the control group (7.16%), and the difference is 6.88%.

Table 2. Average weight gain percentage for control group and test group.

Date	Control/test group	Quantity	Average weigh (kg)	Average weight gain (%)
2016/08/12	control	6	39.98	
	test	6	41.61	
2016/08/13~09/12	control	6	45.40	13.48
	test	6	46.47	11.80
2016/09/13~10/12	control	6	50.03	10.16
	test	6	51.67	11.21
2016/10/13~11/12	control	6	54.68	9.27
	test	6	54.85	6.14
2016/11/13~12/12	control	6	58.70	7.16
	test	6	62.53	14.04

4.2. T-test results

The t-test results indicate that in the first three months, there is no difference in weight gain between the two groups (Table 3). However, since the fourth month, when adding the *Ganoderma lucidum* compound, the *p* value is significant ($p < 0.05$). There is a significant difference between the two groups.

Table 3. T-test statistical results.

Date	t-test	*p* value
2016/08/13~09/12	0.689	0.506
2016/09/13~10/12	−0.594	0.566
2016/10/13~11/12	1.130	0.285
2016/11/13~12/12	−3.181	0.010*

Note: * means *p* value < 0.05

4.3. Screening results of inflammatory factors

In this study, the inhibitory effects of NO, IL-6, and TNF-α activated by bacterial LPS were used to obtain further evidence for goats' healthy development. This section is aimed at the regulation effects on inflammation-related cytokines. Figure 1 shows that at different concentrations of 25 μg/ml and 50 μg/ml, the inhibition of LPS-induced NO production is 34.0% (100%–66.0%) and 41.3% (100%–58.7%), respectively. The *Ganoderma lucidum* compound can inhibit the NO production induced by LPS by more than 20%, which means that it can inhibit the NO production induced by LPS. Figure 2 shows that the production of IL-6 induced by LPS is 46.4% (100%–53.6%) and 57.1% (100%–42.9%), respectively, at different concentrations of 25g/ml and 50g/ml. Similarly, the *Ganoderma lucidum* compound can inhibit the IL-6 production induced by LPS. However, because the *Ganoderma lucidum* compound can inhibit the TNF-α production induced by LPS by less than 20% (12.9% and 12.7% as showed in Figure 3), it has no effect on the inhibition of TNF-α production induced by LPS. On the other hand, regarding concerns for the survival rate of MTT cells, the *Ganoderma lucidum* compound has shown no significant cytotoxicity on the tested cells as shown in Figures 1, 2, and 3 (diamond dot plot). In other words, in the cell culture medium, the increase in the dosage of the *Ganoderma lucidum* compound does not increase the cell death rate (cell survival rate > 80%).

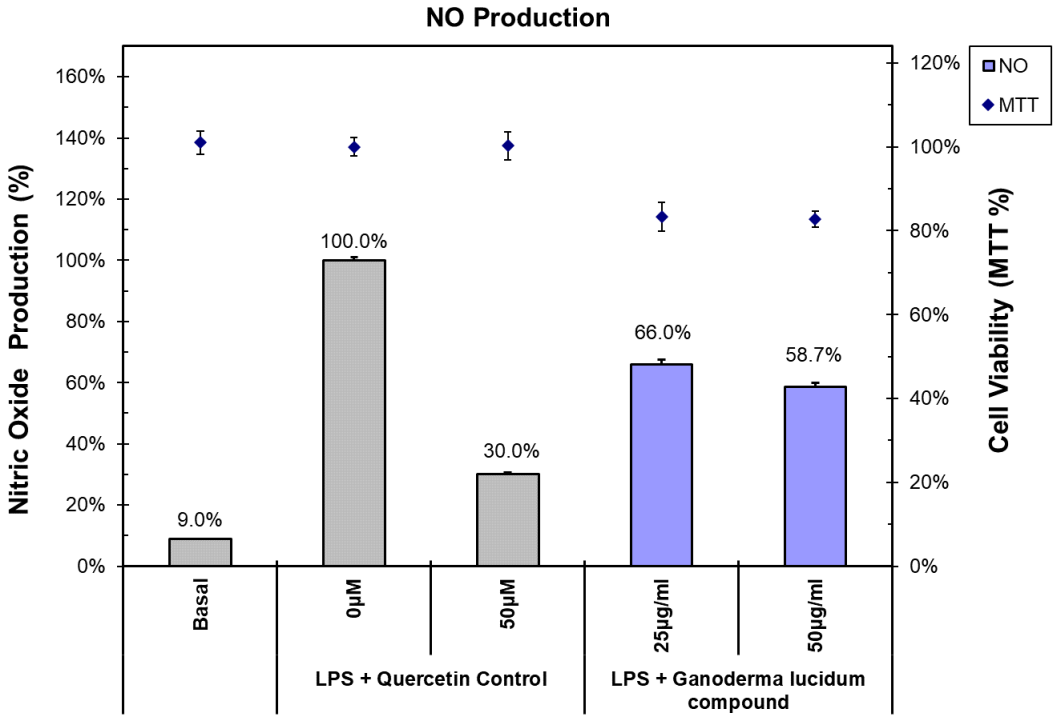

Figure 1. Inhibitory effect of LPS-induced NO production and survival rate of MTT cell.

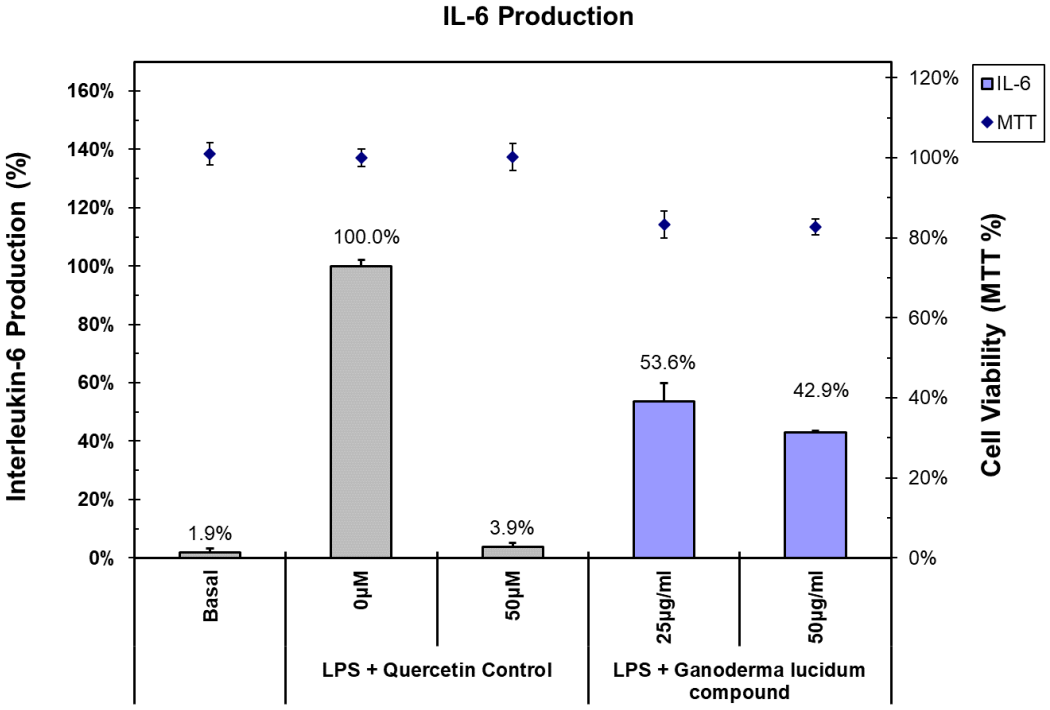

Figure 2. Inhibitory effect of LPS-induced IL-6 production and survival rate of MTT cell.

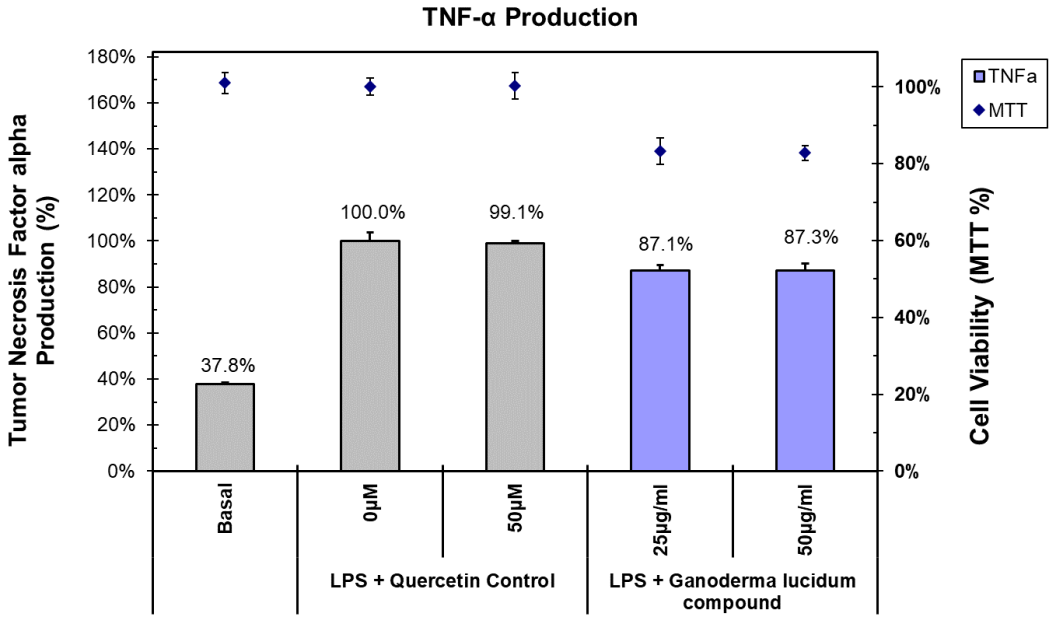

Figure 3. Inhibitory effect of LPS-induced TNF-α production and survival rate of MTT cell.

5. Concluding remarks

5.1. Research findings

Based on a review of the literature on circular agriculture, food production chain, crop planting, livestock farming, goats, and *Ganoderma lucidum* compound, this paper has presented the effects of *Ganoderma lucidum* compound added in feed on goat weight and anti-inflammatory through this case study. Experimental results demonstrate that in the first three months, there was no difference between the control group and the test group according to the t-test. However, in the fourth month, after adding the *Ganoderma lucidum* compound, there was a significant weight gain difference between the two groups. The weight gain difference between the two groups was at 3.666 kg. The average weight gain difference per goat was 6.88%. Moreover, the standard deviation in the test group is 1.703, which is stable compared with 2.252 in the control group. Additionally, by inhibiting NO and IL-6 regular inflammatory production tests, the anti-inflammatory response of the *Ganoderma lucidum* compound to macrophage cell lines activated by bacterial LPS is verified to obtain evidence supporting the health of the goat. Nevertheless, it has no effect on the inhibition of TNF-α production induced by LPS. Furthermore, the concentration of the *Ganoderma lucidum* compound has no significant effect on the survival rate of macrophages by MTT assay, indicating that there is no toxic reaction to the tested cells.

5.2. Managerial implications

The livestock breeding industry have used antibiotics for many years in order to promote the efficiency of growth; however, this long-term business practice has led to the emergence of antibiotic-resistant strains, while the antibacterial medical system for human survival has also brought considerable threats. This study found that dietary supplementation of the *Ganoderma lucidum* compound has better performance in weight gain and growth of goats. It could serve as an alternative to reduce antibiotics use and promote health of goats or even the welfare of livestock. In the face of rapid environmental changes and fierce competition in the market, in order to achieve survival and sustainable development, companies should continue to develop innovative and differentiated green business models, and enhance the added value of products and services.

5.3. Future research directions

In spite of the fact that the sample of this study is limited, the best efforts have been made on the research topic of the weight gain test, hoping to help improve the operating management efficiency of the livestock owners. Although this study represents a good start for research on this area, it also suffers certain limitations. Certain topics merit further investigation. First, a natural extension of this study is to consider additional case studies and increase the number of test samples for future research. Second, the analysis of blood traits can be added in the future to test their growth and immune regulation effects. Third, the simultaneous comparison of weight difference between *Ganoderma lucidum* compound and antibiotics can be considered. Fourth, whether the difference in

weight gain comes from the effect of *Ganoderma lucidum* or the effect of the compound needs to be clarified. Fifth, in order to fully reflect the cost of feed, it is necessary to recalculate the cost due to weight gain. Additionally, feeding the *Ganoderma lucidum* compound not only can promote weight gain and improve animal health and welfare, but also reduce maintenance, medicine and other costs. Therefore, if these costs can be calculated, then the research benefits will be more accurately expressed.

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