International Journal of Science Academic Research
Vol. 03, Issue 12, pp.4770-4774, December, 2022
Available online at http://www.scienceijsar.com

ISSN: 2582-6425

Research Article

EFFECTS OF DIETARY SUPPLEMENTATION OF CHITOSAN OLIGOSACCHARIDE ON THE GROWTH PERFORMANCE, BIOCHEMICAL INDEXES AND IMMUNE FUNCTION OF WEANED LAMBS

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Received 10th October 2022; **Accepted** 16th November 2022; **Published online** 12th December 2022

Abstract

The current study was aimed to explore the effects of chitosan oligosaccharide (COS) on the growth performance, blood biochemical indexes and immune function of weaned lambs. A total of forty healthy weaned lambs of Dorset F2 generation with similar body weight were selected and randomly divided into four groups. The basic diet was supplemented with 50 mg/kg, 100 mg/kgand 150 mg/kgCOS, and a blank control group was also set, with the test periodof 42 days.The results showed that the final body weight and ADG of Groups Ⅱ andⅢ were significantly higher than those in the control group. In contrast, the FCRs of Groups Ⅱ andⅢ were significantly lower than those in the control group. The alkaline phosphatase activity and cholesterol content of Groups Ⅱ and Ⅲ were significantly lower than those in the control group. The immunoglobulin A content of Groups Ⅱ andⅢ was significantly higher than that in the control group, and the immunoglobulin G content of Groups Ⅱ andⅢ was significantly higher than that in the control group. The content of tumor necrosis factor-alpha in Groups Ⅱ and Ⅲ was significantly higher than that in the control group. The addition of COS to the diet of weaned lambs can improve their growth performance and blood biochemical indexes and enhance the body'simmunity, and the optimum supplement of dietary COS level should be 100 mg/kg.

Keywords: Feed conversion ratio, Alkaline phosphatase, Pathogenicityrate.

INTRODUCTION

Chitosan (CTS), the product of chitin after deacetylation, is the only cationic animal fiber and alkaline polysaccharide discovered. Due to its various physiological functions (Jeno YJ et al, 2000), such as hypolipidemic effects, bacteria resistance, bacteriostasis, immunity enhancement and oxidation resistance, it has been widely supplemented to the diet. However, CTS has a larger molecular weight and is insoluble in water and alkaline solution. Its development and application are greatly restricted. Chitosan oligosaccharide (COS) is a water-soluble CTS with low molecular weight after CTS degradation. Itretains the natural characteristics of CTS macromolecules. The CTS with different ranges of molecular weight has unique physiological functions. The pharmacological activity of COSis14times that of CTS with the same weight (Lintao Zeng *et al.,* 2007), significantly reducing the supplemental CTS level. Therefore, as a new type of green additive, COS has attracted more attention in the field of livestock and poultry production (Yuqiang Luo *et al.,* 2009). Since the development of gastrointestinal flora in animals before and after weaning is very unstable, pathogenic bacteria can easily multiply in the intestine, which is an important reason for animal's susceptibility to diseases, such as diarrhea. In the process of changing from breastfeeding to forage feeding during the weaning period, symptoms are appeared due to the change of micro ecological environment in the digestive tract, leading to problems in the growth and development of weaned lambs and their intestinal health, which causes serious economic losses to culturists. As a kind of green feed additive, COS has been widely studied in pig and chicken breeding, but there is limited information on its application in weaned lamb breeding.

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Thus, the current study aimed to examine the effects of COS on the growth performance, blood biochemical indexes and immunity of weaned lambs and investigate the optimum additive level of COS to provide data support for its reasonable application in weaned lamb breeding.

MATERIALS AND METHODS

Test materials

COS (chitosan oligosaccharide, purity >95%) was purchased from Jinan Xinhong Chemical Co., Ltd., Jinan City, Shandong, China.

Experimental animals and feeding management

Forty 45-day-old healthy weaned lambs with similar body weight were selected as experimental animals, which were provided by a breeding farm of Xinjiang Western Animal Husbandry Co., Ltd., Xinjiang, China. The experiment was carried out in a pen house, with routine feeding management, free drinking and regular free-feeding conducted for the whole period. The pens were cleaned every day and disinfected regularly.

Experimental design

The experiment was designed as a single-factor completely randomized experiment, and the selected 40 lambs were completely randomly divided into 4 groups, with 10 lambs in each treatment group. The test period was 42 days, including 7 days of the pilot test and 35 days of the formal test. The lambs in the control group were fed with a basic diet, while the lambs were fed with a diet containing 50 mg/kg (Group I), 100 mg/kg (Group II) and 150 mg/kg (Group III) of COS, respectively.

The basic diet composition and nutritional level of the experimental lambs are listed in Table 1.

Table 1 Composition and nutritional level of basic diet

Composition of raw materials	Contents /%	Nutritional level	
Corn	32.20	Dry matter%	86.89
Soybean meal	5.50	Metabolic energy/(MJ/kg)	12.45
Alfalfa licorice	21.45	Crude protein/%	17.98
Corn silage	33.70	Crude fat/%	2.25
The cotton seed shell	5.00	Neutral detergent fiber/%	16.02
Calcium hydrogen phosphate	1.15	Acid detergent fiber /%	24.75
Premix	1.00	Calcium/%	1.34
Total	100.00	Phosphorus/%	0.55

Note:1.Onekilogramofpremixcontains100000IU of VA,300000IU of VD,1800 mg of VE, 350 mg of nicotinic acid,2g of Zn, 0.32g of KI, 25g of $FeSO_4 \cdot 7H_2O$, 4g of $CuSO_4 \cdot 5H_2O$, 0.06g of $CoCl_2 \cdot 6H_2O$, 15g of $MnSO_4 \cdot 5H_2O$, 18g of $ZnSO_4 \cdot 7H_2O$and0.04g of Na_2SeO_3; 2. In the nutrition level, metabolic energy is a calculated value, and others are measured values.

Determination indicators and methods

Growth performance: During the start and end of the experiment, the weight of the lambs was accurately weighed after the lambs were fasted for 12 hours, and the daily feed intake of lambs in each group was accurately recorded. The feed intake data were used to calculate the average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) of lambs, and the calculation formulas were as follows:

Average daily gain (ADG) = (final weight-initial weight)/test days
Average daily feed intake (ADFI)= total feed intake during the test period/test days
Feed conversion ratio = average daily feed intake (ADFI)/average daily gain (ADG)

Blood biochemical indexes: On day 35 of the experiment, four lambs were selected from each group to collect 10 ml blood from the jugular vein, which was centrifuged at 3500 r/min for 10 minutes. The upper serum was taken to detect serum biochemical indexes, such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), urea nitrogen (UN), total protein, cholesterol, etc. An automatic biochemical analyzer was employed for the detection. The kits were purchased from Sino Best Bio Co., Ltd., Xingtai, China. The rest of the serum was stored at -20℃ for detecting immune indexes.

Immunity indexes: The immunity indexes of blood samples collected on day 35 of the experiment included immunoglobulin A (IgA), immunoglobin G (IgG) and immunoglobin M (IgM), interleukin -6 (IL-6), interleukin -2 (IL-2) and tumor necrosis factor-α (TNF-α). The above immunity indexes were detected by enzyme-linked immunoassay (ELISA), and the kits were purchased from Sino Best Bio Co., Ltd., Xingtai, China. The detection was conducted according to the kit instructions.

Incidence of lambs: The incidence of all lambs in each group was observed and recorded.

Data analysis

The data recorded in the current study were analyzed by Excel, and SPSS 20.0 statistical software was used for the one-way analysis of variance (ANOVA) test. The multiple comparisons of data among groups were conducted by the Duncan's method. The results were expressed as mean ± standard deviation (SD), and $P<0.05$ was used as the standard to determine whether the difference was significant.

RESULTS

Effects of different levels of COS on growth performance of weaned lambs

As shown in Table 2, there was no significant difference in initial body weight and ADFI of lambs among the treatment groups ($P>0.05$). The final body weight and ADG of Groups Ⅱ and Ⅲ were significantly higher than those of the control group ($P<0.05$), which increased by 8.90% and 7.89% and 32.82% and 28.34%, respectively. The final weight and ADG of Group I were higher than those of the control group, but the difference was not significant ($P>0.05$). The FCR of Groups Ⅱ and Ⅲ was significantly lower than that of the control group ($P<0.05$), which was decreased by 24.53% and 25.43%, respectively. The feed weight of Group I was slightly lower than that of the control group, with no significant difference ($P>0.05$).

Effects of different levels of COS on blood biochemical indexes of weaned lambs

According to Table 3, there was no significant difference in the activities of ALT, AST and creatinine in the serum of weaned lambs among the treatment groups ($P>0.05$). No significant difference was observed in the contents of albumin, total protein, UN, triglyceride (TG) and blood glucose ($P>0.05$).The activity of ALP in Groups Ⅱ and Ⅲ was significantly lower than that in the control group ($P<0.05$), which was decreased by 18.73% and 16.79%, respectively. The activity of ALP in the serum of Group I was lower than that in the control group, but the difference was not significant ($P>0.05$). The cholesterol content of Group Ⅱ and Ⅲ was significantly lower than that of the control group ($P<0.05$), which was decreased by 13.77% and 12.30%, respectively. The cholesterol content of Group I was slightly lower than that of the control group, with no significant difference ($P>0.05$).

Effects of different levels of COS on immune indexes of weaned lambs

There was no significant difference in the contents of IgM, IL-2 and IL-6 in the serum of weaned lambs among different treatment groups ($P>0.05$) (Table 4). IgA contents of Groups Ⅱ and Ⅲ were significantly higher than those of the control group ($P<0.05$), which increased by 9.84% and 10.78%, respectively, while the IgA content of Group I was slightly higher than that of the control group, with no significant difference ($P>0.05$). IgG contents of Groups I, II and III were significantly higher than those of the control group ($P<0.05$), which increased by 15.97%, 23.16% and 24.85%, respectively. TNF-α contents in Groups II and III were significantly higher than those in the control group ($P<0.05$), which increased by 16.48% and 15.07%, respectively. TNF-α content in Group I was slightly higher than that in the control group, with no significant difference ($P>0.05$).

Measurement of the incidence of lambs

As shown in Table 5, four lambs in the control group presented diarrhea symptoms, with the highest incidence rate of 40% in the control group and 10% in Group I. There were no sick lambs in Groups II and III.

Table 2 Effects of different levels of COS on growth performance of weaned lambs

Items	Control group	Group Ⅰ	Group Ⅱ	Group Ⅲ
Initial body weight /kg	8.63±0.172	8.68±0.163	8.53±0.113	8.58±0.132
Final body weight /kg	12.65±0.223	13.01±0.254	13.87±0.203	13.73±0.192
Average daily gain(ADG, kg/d)	0.11±0.012	0.12±0.013	0.16±0.034	0.15±0.023
Average daily feed intake(ADFI, g/d)	0.28±0.072	0.29±0.043	0.28±0.054	0.27±0.033
Feed conversion ratio (FCR)	2.22±0.243	2.13±0.192	1.67±0.163	1.65±0.183

Note: Different uppercase letters indicate a significant difference (*P*<0.05).

Table 3. Effects of different levels of COS on blood biochemical indexes of weaned lambs

Items	Control group	Group Ⅰ	Group Ⅱ	Group Ⅲ
Alanine aminotransferase (U/L)	23.87±2.152	22.24±1.883	21.73±2.022	22.67±2.163
Glutamate transaminase (U/L)	89.32±10.223	88.10±10.003	88.33±9.473	87.72±9.822
Alkaline phosphatase (U/L)	436.16±87.333	432.84±80.194	420.96±76.032	424.84±76.143
Albumin (g/L)	32.39±2.453	31.77±1.962	31.17±1.853	31.43±2.033
Total protein (g/L)	64.23±1.663	66.21±1.692	68.03±1.883	67.73±2.033
Urea nitrogen (mmol/L)	5.26±1.092	5.01±1.113	4.94±0.923	5.12±1.194
Triglyceride (mmol/L)	0.32±0.413	0.32±0.414	0.27±0.372	0.29±0.343
Cholesterol (mmol/L)	2.01±0.072	1.84±0.063	1.73±0.033	1.76±0.042
Blood glucose (mmol/L)	3.63±1.023	3.49±1.042	3.47±1.113	3.44±1.203
Creatinine (μmol/L)	52.10±4.133	50.65±4.012	50.26±3.843	49.74±3.823

Note: Different uppercase letters indicate a significant difference (*P*<0.05).

Table 4. Effects of different levels of COS on immune indexes of weaned lambs

Items	Control group	Group Ⅰ	Group Ⅱ	Group Ⅲ
IgA(g/L)	2.11±0.113	2.26±0.112	2.32±0.134	2.34±0.143
IgM(g/L)	1.04±0.082	1.07±0.063	1.09±0.094	1.10±0.102
IgG(g/L)	12.61±1.334	14.79±1.393	15.77±1.512	16.00±1.623
IL-2(ng/L)	53.24±3.203	54.69±3.332	53.01±3.013	54.23±3.173
IL-6(ng/L)	65.90±3.643	64.85±3.012	63.82±3.142	63.46±3.493
TNF-α (ng/L)	421.68±67.113	428.35±63.892	451.35±60.91	442.17±61.403

Note: Different uppercase letters indicate a significant difference (*P*<0.05).

Table 5. The incidence of lambs

Groups	Quantity of sick lambs	Quantity of healthy lambs	Incidence %
Control group	4	6	40
Group Ⅰ	1	9	10
Group Ⅱ	0	10	0
Group Ⅲ	0	10	0

DISCUSSION

Effects of different levels of COS on growth performance of weaned lambs

ADG, ADFI and FCR are the important indexes to evaluate the growth performance of livestock and poultry. There are few reports on the effects of COS on the growth performance of weaned lambs. COS can promote the reproduction of beneficial bacteria, improve the environment in the intestinal tract and promote the digestion and absorption of feed, thereby improving the production performance of animals (Tao Sun *et al.,* 2012). The addition of 0.05% and 0.07% COS to weaned piglets' diets could significantly increase ADG and ADFI, reduce FCR of piglets, relieve weaning stress of piglets, and play an active role in improving feed utilization rate and promoting the growth of piglets (Lixin Yao, 2011). Hu *et al.* (2018) found that the addition of 50 mg/kg low-molecular-weight CTS to the diet can effectively improve the growth performance of weaned piglets, improve the intestinal barrier function, and reduce intestinal inflammation. Goirietal. (2010) discovered that CTS could regulate rumen and cecum fermentation characteristics of sheep to improve feed digestibility. Pereira *et al.* (2018) indicated that the addition of CTS to the diet of fattening lambs could improve the nitrogen balance, microbial protein synthesis and the digestibility of CP, DM and NDF, enhancing the production performance of lambs. Tang *et al.* (2011) reported that the addition of COS to the diet could improve the production performance of weaned piglets, reduce diarrhea rate, inhibit the growth of intestinal pathogenic bacteria and promote the growth of beneficial bacteria. Sun et al. (2009) indicated that adding different doses of CTS to the basic diet of dairy calves could increase daily gain and ADFI. The current study showed that the addition of COS in the diet significantly increased the ADG and ADFI of weaned lambs and reduced the FCR. Groups Ⅱ and Ⅲ presented better experimental effects, which is consistent with the above research results. COS could improve the growth performance of animals because it could improve the intestinal environment of animals, thus enhancing the body's immunity and promoting the digestion and absorption efficiency of nutrients by animals.

Effects of different levels of COS on blood biochemical indexes of weaned lambs

Blood is a major component of the circulatory system of animals. Serum biochemical indicators reflect the health status of animals and the metabolism of animals (LIU *et al.,* 2015).In the current study, the addition of COS in the diet of weaned lambs had no significant effect on the activities of ALT, AST and creatinine, and the contents of albumin, total protein, UN, TG and blood glucose. The indicator values were close to those of the control group. The results are similar to the research finding by Qiao *et al.* who found that the addition of different levels of COS exhibited no significant influence on the contents of total protein, albumin and globulin in serum (Lihong Qiao *et al.,* 2013). Tang *et al.* (2005) found that the addition of 0.025% CTS to the diet could reduce the levels of serum hematuria nitrogen, total cholesterol and total TG and increase the total protein content, which is different from our results. It may be related to the molecular weight and the supplemental COS level and experimental animals, and its specific mechanism needs further investigation. In the current study, ALP activity and cholesterol content in Groups II and III were significantly lower than those in the control group, which is similar to Qiao's (2013) research findings in which cholesterol content in the diet supplemented with 50 mg/kg or 100 mg/kg COS was significantly reduced. Min Tang's (2009) research observed that the ALP content in the serum of weaned piglets was increased with different additive levels of COS, which may be caused by different additive levels of COS. The specific mechanism needs further study. The results showed that COS had no significant effect on liver and kidney indexes of weaned lambs and had no negative effects on the liver and kidney of weaned lambs. COS, as a natural green additive, could be applied in weaned lamb breeding and production, which has no negative effect on animal liver and kidney and other internal organs.

Effects of different levels of COS on immunity indexes of weaned lambs

IgA, IgG and IgM in animals are the main antibodies that mediate humoral immunity, which can prevent pathogens from invading the first line of defense in the animal body, activate complement and neutralize toxins (FATHI *et al.,* 2017). Cytokines play a key role in the organism's immune response reaction, inflammatory response, hematopoiesis, tissue repair, embryogenesis, growth, development, etc. (Wei Yuan *et al.,* 2015). COS addition in the daily diet can protect the structure and function of the intestinal tract and improve intestinal immunity through reducing pro-inflammatory cytokines and increasing the expression of anti-inflammatory cytokines (Neimert Andersson *et al.,* 2011; Bueter *et al.,* 2014). The current study showed that the addition of a certain level of COS to weaned lambs' diet could increase the contents of IgA, IgG and TNF-α in serum, suggesting that COS could significantly improve the immunity of animals. Xiao et al. (2011) added different doses of CTS to weaned piglets' diets, which could improve the levels of IgM, IgG and IgA in piglets' serum and the relative expression levels of interleukin-1β (IL1B), IL-6 and TNF-α in ileum mucosa . Li et al. (2014) indicated that CTS could increase IgM and IgG levels in piglet serum and enhance the relative expression of IL-1, IL-6 and TNF- α mRNA in ileum mucosa. With the increase of CTS concentration, the expression presented an increasing tendency, and the nonspecific immunity of piglets could be promoted. COS can improve the immunity of animals and the immunomodulatory process. COS participates in immune cytokines, such as IL-1, IL-6 and TNF-α, which are important cytokines to ensure the normal immune function, enhancing the immune function of animals.

Effects of different levels of COS on the incidence of weaned lambs

CTS is the growth factor of *Bifidobacterium* and *Lactobacillus*, which can promote their proliferation and inhibit the reproduction of harmful bacteria to regulate intestinal microflora (Wan Ngah W S et al,2014).In the current study, the overall health level of lambs in the experimental groups was better. When the content of COS reached more than 0.02%, the disease protection rate of lambs in the experimental groups could reach 100%.Ren et al. (2008) indicated that the addition of 0.1% CTS to the diet of dairy cows could change the intestinal flora, significantly increase the number of *Lactobacillus* and reduce the number of *Escherichia coli*. Xiao *et al.* (2012) discovered that the expression of occludin (OCLN) and tight junction protein (ZO-1) in jejunum mucosa of the CTS group increased, and the addition of dietary 300 mg/kg CTS can reduce the damage of intestinal mucosal barrier function in early-weaned piglets. Xu *et al.* (2013) added different levels of CTS to the basic diet. The results indicated that the total numbers of aerobic bacteria and *E. coli* in the cecum and colon of the CTS supplemented group was lower than those of the control group, while the number of lactic acid bacteria was higher than that of the control group. The results demonstrated that the addition of CTS could effectively inhibit the proliferation of harmful bacteria in the intestinal tract, promote the reproduction of beneficial bacteria, improve the environment in the intestinal tract, enhance the mechanical barrier function of the animal intestinal mucosa and reduce the incidence of animal diseases, then promoting the growth and development of piglets.

Conclusion

The addition of a certain level of COS to thediet of weaned lambs can improve the growth performance, blood biochemical indexes and immunity of weaned lambs. In the current study, the effects of COS on the growth performance, immunity and disease resistance of weaned lambs were investigated to providea theoretical basis for the application of COS in the healthy breeding of lambs and lay a foundation for the study of the mechanism of COS. Based on the results and cost factors, the optimum supplement of dietary COS level should be 100 mg/kg for weaned lambs.

Acknowledgements

This research was supported by the National Natural Science Foundation of China (NSFC)(grant no.31360558).

Author contributions

Conceived and designed the project: Pan, X.L. Collected data and performed the experiments: Zhang, E.H. Conceived and conducted the data analysis: Wang,M. Wrote the paper: Zhang, E.H. All authors read and approved the fnal manuscript.Meng Wang and Erhong Zhang are Co-first authors.

Conflict of interest

The authors declare there are no conflicts of interest.

Ethical standards

Not applicable.

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