

## Effects of Chitosan on Serum Lipids Index in Chinese Holstein Dairy Calves

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**Abstract:** An experiment was conducted to study the effects of different doses of chitosan on growth and serum parameters in dairy calves. A total of 20 and 10 days old dairy calves were randomly allotted to four dietary treatments. The experiment period were 30 days. The control dairy calves were fed the basal diet alone, the other treatments fed the basal diet supplemented with 200, 400, 600 mg day<sup>-1</sup> Chitosan (CHS), respectively. In the blood serum biochemistry target aspect, the total cholesterol content significantly decreased for the 600 mg day<sup>-1</sup> Chitosan (CHS) (p<0.05). In 20th and 30th, high density lipoprotein content significantly decreased for the 600 mg day<sup>-1</sup> Chitosan (CHS) (p<0.05).

**Key words:** Chitosan, total cholesterol, total triglyceride, high density lipoprotein, low density lipoprotein, Chinese holstein dairy calves

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### INTRODUCTION

Chitosan is also called amino polysaccharide or deacetylchitin, the chemical name S-(1, 4)-2-amino-2-deoxy-D-glucan by chitin deacetylated generated after a basic polysaccharide of natural origin is a natural product of nature after cellulose production. Shell is widely found in some of the lower animals, especially arthropods (insects, crustaceans, etc.) as well as fungi, algae, yeast cell wall is a natural polymer active straight-chain polysaccharide. It has a non-toxic, no side effects, pollution-free environment biodegradability as well as good organizational compatibility characteristics. The chitosan was originally applied to the field of medicine, food, cosmetics, agriculture and for people relatively large. But, its as feed additives domestic their research has just begun. Existing experimental studies (Nie *et al.*, 2004; Zhu *et al.*, 2003; Lim *et al.*, 1997; Nishimura *et al.*, 1984; Seferian and Martinez, 2000) show that chitosan has antibacterial effects, may improve intestinal road microbial environment and may enhance the regulation of immune function in animals. Studies (Kobayashi *et al.*, 1996; Razdan and Pettersson, 1994; Razdan *et al.*, 1997; Gallaher *et al.*, 2000) reported that chitosan can reduce ileal fat digestibility and can effectively reduce blood cholesterol and triglyceride levels. In addition, there are reports (Ma *et al.*, 2001; Huang, *et al.*, 2007; Tang *et al.*, 2005) that the chitosan to promote animal

growth performance. Therefore, chitosan as a natural activity of polysaccharide wants to be novel alternatives to antibiotics.

Chitosan also known as deacetylation of chitin is the only alkaline active polysaccharides that occur in nature. Currently, most researchers believe that chitosan has a regulating immunity, antimicrobial activity widely used for medicinal and health food. In recent years, animal production also gradually increased. However, so far no information chitosan of milk calf serum cholesterol, triglycerides, high-density lipoprotein and low-density lipoprotein levels, the impact of research reports, the test on the basis of previous studies from chitosan on calf blood biochemical indicators to explore its pro-growth mechanism and filter out the appropriate amount of add, aims to provide a theoretical basis for chitosan as alternatives to antibiotics in dairy production.

### MATERIALS AND METHODS

**Animals:** The experiment began on March 2010. Twenty Holstein dairy calves were provide by Great Wall dairy Inc. (Zhangjiakou) (<http://3813914.71ab.com/prod.asp>).

**Experiment design:** With complete random design, twenty calves of 10 days of age were assigned to four

dietary treatments (control, treatment groups I, II, III) at random. The trial period was 30 days. Chitosan was supplemented in basal diet at 0, 200, 400, 600 mg day<sup>-1</sup>, respectively used to treat the four groups.

**Management:** Animals were managed according to conventional protocol including feed stuff, feed, management and daily arrangement. Each pen holds one animal. Water was provided *ad libitum*. Clear environment was maintained.

**Determination of indicators:** Blood samples were collected on days 10, 20 and 30 from jugular vein. Samples were centrifuged at 3000 rpm for 15 min (type 800 centrifuge, Shanghai). -20°C cryopreservation determination of the following indicators: Triglycerides (TG) Enzymatic Colorimetric Method (GPO-PAP) was measured by (GPO-PAP), determination of High-Density Lipoprotein (HDL) Enzymatic Colorimetric Method (GPO-PAP) was measured; Total Cholesterol (TC); Low-Density Lipoprotein (LDL) using Glycerol Phosphate Oxidase- Peroxidase (GPO-PAP) determination.

**Statistical analysis:** Single factor ANOVA analysis was done with SAS. Results were expressed as mean±SD. Treatments were considered significant difference at different values.

**RESULTS**

**Effects of chitosan on TC in dairy calf:** Calf serum biochemical indicators of additive chitosan shown in the Table 1. Table 1, adding chitosan each group than the control group, reducing the content of the total serum cholesterol and this test the effect is more apparent with the increase of the number of days and amount of chitosan test; in 10, 20 and 30 days, 600 mg day<sup>-1</sup> chitosan group compared to the control group was significantly reduced serum TC concentration (p<0.05).

**Effects of chitosan on TG in dairy calf:** Table 2, the addition of chitosan calf serum triglyceride levels, the impact is not significant (p>0.05) but each dose group are reduced with the extension of the feeding time.

**Effects of chitosan on HDL in dairy calf:** Table 3 shows that the group I and II compared with the control group, can improve the content of calf serum high-density lipoprotein (p>0.05); III group at 20 and 30 days compared with the control group, reduced calf serum high-density lipoprotein levels (p<0.05)

**Effects of chitosan on LDL in dairy calf:** Table 4 showed data from the experiment, II group than the control group,

Table 1: Effects of chitosan on TC in dairy calf (mmol/L)

Groups	10 days	29 days	30 days
Control group	3.59±0.37 <sup>a</sup>	3.67±0.09 <sup>a</sup>	3.56±0.38 <sup>a</sup>
I	3.54±0.12	3.44±0.28	3.41±0.14
II	3.49±0.23	3.39±0.50	3.40±0.13
III	2.28±0.54 <sup>b</sup>	2.19±0.65 <sup>b</sup>	1.98±0.51 <sup>b</sup>

Table 2: Effects of chitosan on TG in dairy calf (mmol/L)

Groups	10 days	20 days	30 days
Control group	0.18±0.02 <sup>a</sup>	0.19±0.06 <sup>a</sup>	0.18±0.01 <sup>a</sup>
I	0.18±0.08 <sup>a</sup>	0.19±0.03 <sup>a</sup>	0.16±0.02 <sup>a</sup>
II	0.18±0.08 <sup>a</sup>	0.19±0.01 <sup>a</sup>	0.16±0.04 <sup>a</sup>
III	0.18±0.02 <sup>a</sup>	0.18±0.08 <sup>a</sup>	0.15±0.02 <sup>a</sup>

Table 3: Effects of chitosan on HDL in dairy calf (mmol/L)

Groups	10 days	20 days	30 days
Control group	1.61±0.26 <sup>a</sup>	1.63±0.21 <sup>a</sup>	1.63±0.27 <sup>a</sup>
I	1.86±0.27 <sup>a</sup>	1.84±0.29	1.89±0.19
II	1.84±0.20 <sup>a</sup>	1.92±0.30	1.94±0.16
III	1.10±0.01 <sup>a</sup>	1.08±0.24 <sup>b</sup>	1.03±0.02 <sup>b</sup>

Table 4: Effects of chitosan on LDL in dairy calf (mmol/L)

Groups	10 days	20 days	30 days
Control group	1.63±0.68 <sup>a</sup>	1.65±0.56 <sup>a</sup>	1.63±0.33 <sup>a</sup>
I	1.73±0.24 <sup>a</sup>	1.70±0.23 <sup>a</sup>	1.69±0.27 <sup>a</sup>
II	1.60±0.51 <sup>a</sup>	1.58±0.08 <sup>a</sup>	1.56±0.37 <sup>a</sup>
III	1.02±0.45 <sup>b</sup>	0.88±0.44 <sup>b</sup>	0.97±0.68 <sup>b</sup>

The same columns in different the shoulder tables letters indicate significant difference (p<0.05), the same letter indicates no significant difference

reduced calf serum LDL levels (p>0.05); III group compared with the control group, significant reduced calf serum LDL levels (p<0.05).

**DISCUSSION**

Shang *et al.* (2006) reported that the application of 6 g day<sup>-1</sup> chitosan can reduce the serum cholesterol content in lactating dairy cows. Razdan and Pettersson (1994) reported that 30 g kg<sup>-1</sup> chitosan added to broiler diets, test 10 and 18 days of serum total cholesterol, HDL-cholesterol content decreased but no significant change in triglyceride content, ileal fat digestibility decreased by 26%. Wu *et al.* (1994) test showed that elevated serum total cholesterol can be effectively suppressed which reduces the effect mainly of the total cholesterol in lowering LDL cholesterol and VLDL cholesterol but a certain dose to rats intake chitosan enables high-density lipoprotein cholesterol increased. For the mechanism of chitosan lower serum cholesterol, Kanauchi *et al.* (1994) and gastric acid in the stomach after oral chitosan gel formation such a gel does not decompose in the intestines can be maintained within the pH range. This gel adsorption can adsorb bile acids and cholesterol excreted through feces, thereby reducing fat and cholesterol in the blood, reduce fat absorption, thereby reducing the level of serum total cholesterol and triglycerides.

The possible mechanism of chitosan interfere with fat digestion, many researchers from research to explore: chitosan combined with dietary fat, block fat digestion and absorption. Kanauchi *et al.* (1995) by means of the *in vitro* and *in vivo* combined found that chitosan can be combined with the fat in the diet and to prevent the fat from being absorbed in the intestine was found in *in vitro* tests, the chitosan can be combined with the equivalent of 4-5 times its own weight of fat, formed chitosan-fat complexes but the strong binding ability of the bile salt. Reduce the activity of the lipase, reduce fat absorption. Lipase plays a vital role in the digestion and absorption of fat. Kobayashi *et al.* (2002) study found that diets supplemented with chitosan significantly reduce the activity of lipase per gram of intestinal contents, thereby reducing fat absorption. Interference fat bile acid enterohepatic circulation. Since, the chitosan component of polyethylene glucamine chains with tetravalent ammonium ion with a high anion exchange performance and capable of binding bile acids, to prevent the circulation of bile acids to reduce fat absorption, increasing the amount of discharge of the fecal fat. The impact of the trial of chitosan on triglycerides is inconsistent with the results of other studies. Add 30 g kg<sup>-1</sup> chitosan to broiler diets but Razdan and Pettersson (1994) reported that in test 10 and 18 days of serum total cholesterol, HDL-cholesterol content decreased but no triglyceride content significant changes in the same. Its mechanism of action is for further study.

Chitosan can reduce Low-Density Lipoprotein (LDL) and increased High-Density Lipoprotein (HDL) with two-way adjustment (Xu, 2004), further study of mechanism of action, reduce LDL, the extent and degree of deacetylation relative molecular weight of the sample. From the test data shows the low-dose, chitosan increased High-Density Lipoprotein (HDL), high-dose chitosan reduced High-Density Lipoprotein (HDL). With the the chitosan dose increases and the extension of time, chitosan can reduce the ability of Low-Density Lipoprotein (LDL), the stronger. Its mechanism of action has yet to be studied further.

HDL-C lowering in the human body is a danger signals produce atherosclerosis. HDL-C can be used as the the lecithin-turn acyl enzyme substrate, assume the role of the transporter of cholesterol and lecithin if the decrease in HDL-C will affect the plasma lipoprotein cleared is considered to be a risk factor in the clinical conversely, high HDL-C is considered to be advantageous (Chen, 1994). The cholesterol-free diet fed rats longer time, addition of 0.5% chitosan although not exhibit the role of lowering blood cholesterol but found

that there are more cholesterol distribution in the high density lipoprotein in the feed while the very low. The less density lipoprotein (Uchida *et al.*, 1978). Significantly, lower than the control in the mouse plasma HDL-C/TC given steroids feed when given steroids foods while the add services chitosan the HDL-C/TC values rise 7.5% chitosan HDL-can C/TC almost back to the level of the blank control group (LeHoux and Grondin, 1993).

Serum total cholesterol, triglycerides, two important indicators reflect the normal function of the animal's body lipid metabolism, chitosan group compared with the control group, a significant reduction in serum cholesterol, promote the body lipid metabolism. This is consistent with some of the researchers' results.

## CONCLUSION

The trial of chitosan on serum Total Cholesterol (TC), Triglyceride (TG), High Density Lipoprotein (HDL) and Low Density Lipoprotein (LDL) impact, mainly to get the following conclusions: chitosan can reduce serum TC concentration 600 mg day<sup>-1</sup> the chitosan can significantly reduce serum TC concentration ( $p < 0.05$ ); no significant changes in TG content; 200 and 400 mg day<sup>-1</sup> chitosan can increase serum HDL levels trend ( $p > 0.05$ ) but the 600 mg day<sup>-1</sup> chitosan at a later stage can significantly reduce serum HDL levels ( $p < 0.05$ ); 600 mg day<sup>-1</sup> the chitosan can significantly reduce serum LDL levels ( $p < 0.05$ ).

## REFERENCES

- Chen, Q., 1994. Biochemistry. 3rd Edn., People's Medical Publishing House, Beijing, China, Pages: 254.
- Gallaher, C.M., J. Munion, R. Hesslink, J. Wise and D.D. Gallaher, 2000. Cholesterol reduction by glucomannan and chitosan is mediated by changes in cholesterol absorption and bile acid and fat excretion in rats. *J. Nutr.*, 130: 2753-2759.
- Huang, R.L., Z.Y. Deng, C.B. Yang, Y.L. Yin and M.Y. Xie *et al.*, 2007. Dietary oligochitosan supplementation enhances immune status of broilers. *J. Sci. Food Agric.*, 87: 153-159.
- Kanauchi, O., K. Deuchi, Y. Imasato and E. Kobayashi, 1994. Increasing effect of a chitosan and ascorbic acid mixture on fecal dietary fat excretion. *Biosci. Biotechnol. Biochem.*, 58: 1617-1620.
- Kanauchi, O., K. Deuchi, Y. Imasato, M. Shizukuishi and E. Kobayashi, 1995. Mechanism for the inhibition of fat digestion by chitosan and for the synergistic effect of ascorbate. *Biosci. Biotechnol. Biochem.*, 59: 786-790.

- Kobayashi, S., X. Wen and S.I. Shoda, 1996. Specific preparation of artificial xylan: A new approach to polysaccharide synthesis by using cellulase as catalyst. *Macromolecules*, 29: 2698-2700.
- Kobayashi, S., Y. Terashima and H. Itoh, 2002. Effects of dietary chitosan on fat deposition and lipase activity in digesta in broiler chickens. *Br. J. Poult. Sci.*, 43: 270-273.
- LeHoux, J.G. and F. Grondin, 1993. Some effects of chitosan on liver function in the rat. *Endocrinology*, 132: 1078-1084.
- Lim, B.O., K. Yamada, M. Nonaka, Y. Kuramoto, P. Hung and M. Sugano, 1997. Dietary fibers modulate indices of intestinal immune function in rats. *J. Nutr.*, 127: 663-667.
- Ma, X.Z., Y. Yang, X.D. Xie and Y.L. Feng, 2001. Effect of chitosan on growth performance and lipid metabolism of male broiler chickens. *Fujian J. Agric. Sci.*, 16: 30-34.
- Nie, X.Z., Y.L. Yin and J.H. He, 2004. Effects of Chitosan on immunity function and serum cholesterol content in broiler. *Feed Rev.*, 10: 1-4.
- Nishimura, K., S. Nishimura, N. Nishi, I. Saiki, S. Tokura and I. Azuma, 1984. Immunological activity of chitin and its derivatives. *Vaccine*, 2: 93-99.
- Razdan, A. and D. Pettersson, 1994. Effect of chitin and chitosan on nutrient digestibility and plasma lipid concentrations in broiler chickens. *Br. J. Nutr.*, 72: 277-288.
- Razdan, A., D. Pettersson and J. Pettersson, 1997. Broiler chicken body weights, feed intakes, plasma lipid and small-intestinal bile acid concentrations in response to feeding of chitosan and pectin. *Br. J. Nutr.*, 78: 283-291.
- Seferian, P.G. and M.L. Martinez, 2000. Immune stimulating activity of two new chitosan containing adjuvant formulations. *Vaccine*, 19: 661-668.
- Shang, C., H. Chen, S. Liu, L. Wang and Y. Tang *et al.*, 2006. The effects of chitosan on the serum cholesterol and protein in cows. *China Herbivores*, 5: 120-122.
- Tang, Z.R., Y.L. Yin, C.M. Nyachoti, R.L. Huang and T.J. Li *et al.*, 2005. Effect of dietary supplementation of chitosan and galacto-mannan-oligosaccharide on serum parameters and the insulin-like growth factor-I mRNA expression in early-weaned piglets. *Domestic Anim. Endocrinol.*, 28: 430-441.
- Uchida, K., Y. Nomura, M. Kadowaki, H. Takase, K. Takano and N. Takeuchi, 1978. Age-related changes in cholesterol and bile acid metabolism in rats. *J. Lipid Res.*, 19: 544-552.
- Wu, J.L., W. Zhu, Y. Wang and S. Chen, 1994. Effect of chitosan on serum lipids in experimental hypercholesterolemic rats. *Acta Nutrimenta Sinica*, 16: 197-199.
- Xu, S., 2004. Advances in application of chitosan and its derivatives. *China Pharm.*, 13: 80-81.
- Zhu, L.X., Z.G. Song, H. Lin and L. Yuan, 2003. Study the effects of chitin on growth and immune function in broilers. *China Feed*, 4: 15-17.