

# Effects of feeding chitooligosaccharide on growth performance, immunity and serum composition in goats

Chowalit Nakthong<sup>1\*</sup>, Sarawut Taksinoros<sup>2</sup> and Witsanu Wongsawaong<sup>2</sup>

<sup>1</sup>Faculty of Veterinary Science, Mahidol University, Nakornpathom, Thailand, 73170

<sup>2</sup>Livestock and wildlife Hospital, Faculty of Veterinary Science, Mahidol University, Kanchanaburi, Thailand, 71150

\*Corresponding author, E-mail address: vscnt@mahidol.ac.th

## Abstract

A total of 15 female goats ( $28.5 \pm 0.5$  kg) were employed to determine the effects of feeding commercial prebiotic (Nuclear C.O.S), a type of chitooligosaccharide (COS) on growth performance, immunity and serum composition. A complete randomized design (CRD) was used in the experiment. Goats were randomly allotted into 3 treatments with 5 replications each treatment (a goat/pen). Diets were formulated to meet or exceed nutrient requirements (NRC, 1998) and COS (Nuclear C.O.S) was added with 3 levels; 0 ppm (control), 1 ppm (LL) and 2 ppm (HL). Blood samples were collected all goats each treatment and four times on day 0, 21, 42 and 63 after feeding. Goats were bled via venipuncture from jugular vein. During treatment periods, there were trends to improve weight gain and average daily gain (ADG), as COS (Nuclear C.O.S) levels were increased in the diets, although it was not statistically significant ( $p>0.05$ ). The other hand, FCR was reduced when COS (Nuclear C.O.S) level LL as compared to control level ( $p>0.05$ ). Cholesterol level in blood was decreased significantly ( $p<0.05$ ) although total protein was significantly difference increased in COS (Nuclear C.O.S) treated groups ( $p<0.05$ ). There were trend towards reducing triglyceride and trend to upward HDL of goats as dietary COS (Nuclear C.O.S) was increased ( $p>0.05$ ). During the overall period, adding high level (HL) improved lymphocyte cell count ( $p>0.05$ ). White blood cell was not significantly reduced when added level of COS (Nuclear C.O.S) ( $p>0.05$ ). In conclusion, nutrients would be utilized more efficiently in goats fed diet containing COS (Nuclear C.O.S) than those fed control diets due to better condition of microbial populations in alimentary tracts.

**Keywords:** Chitooligosaccharide, growth performance, total protein, total cholesterol, goats

# ผลของการเสริมโคโตโอลิโกแซกคลอไรท์ต่อประสิทธิภาพการเจริญเติบโต ภูมิคุ้มกันและส่วนประกอบของน้ำเลือดในแพะ

เขาวลิต นาคทอง<sup>1\*</sup> สรวุฒิ ทักษิณธร<sup>2</sup> วิษณุ วงษ์สว่าง<sup>2</sup>

<sup>1</sup>ภาควิชาเวชศาสตร์คลินิกและการสาธารณสุข คณะสัตวแพทยศาสตร์ มหาวิทยาลัยมหิดล นครปฐม 73170

<sup>2</sup>โรงพยาบาลปศุสัตว์และสัตว์ป่า ปศุปลาน คณะสัตวแพทยศาสตร์ มหาวิทยาลัยมหิดล กาญจนบุรี 71150

\*ผู้รับผิดชอบบทความ E-mail address: vscent@mahidol.ac.th

## บทคัดย่อ

แพะเพศเมียจำนวน 15 ตัว ( $28.5 \pm 0.5$  kg) ถูกนำมาใช้ทดสอบผลการให้สารพรีไบโอติกที่ใช้ทางการค้า (นิวเคลียส C.O.S) ต่อสมรรถภาพการเจริญเติบโต ระบบภูมิคุ้มกันและส่วนประกอบที่สำคัญในน้ำเลือด โดยออกแบบเป็นแบบสุ่มสมบูรณ์ (CRD) แพะจะถูกสุ่มแบบอิสระเป็น 3 การทดลอง และมีการทำซ้ำทั้งหมด 5 ครั้ง โดยแพะแต่ละตัวอยู่คนละคอก โดยให้สูตรอาหารตามความต้องการโภชนาการ (NRC, 1998) และเพิ่ม COS ใน 3 ระดับ คือ 0 ppm (control), 1 ppm (LL) และ 2 ppm (HL) ตัวอย่างเลือดถูกเก็บในวันที่ 0, 21, 42 และ 63 หลังจากเริ่มทดลอง

จากการทดลองพบว่าอัตราเพิ่มเฉลี่ยน้ำหนักตัวต่อวัน (ADG) เพิ่มขึ้น ( $p > 0.05$ ) ซึ่งตรงกันข้ามกับอัตราการเปลี่ยนอาหารมาเป็นน้ำหนักตัว (FCR) มีค่าลดลง ( $p > 0.05$ ) ระดับคลอเรสเตอรอลรวมในกระแสเลือดลดลงอย่างมีนัยสำคัญ ( $p < 0.05$ ) แม้ว่าปริมาณโปรตีนรวมและจำนวนเม็ดเลือดขาวชนิดลิมโฟไซต์จะสูงขึ้น ( $p > 0.05$ ) ก็ตามเมื่อมีการเพิ่ม COS ลงในอาหารที่ใช้เลี้ยงแพะเป็นการเสริมการทำงานของแบคทีเรียในส่วนของเดินอาหารให้ดีขึ้น ซึ่งมีผลทำให้การย่อยสลายของสารอาหารได้ดีขึ้น น้ำหนักตัวเพิ่มมากขึ้น ระบบภูมิคุ้มกันของร่างกายมีแนวโน้มดีขึ้น ช่วยลดเวลาการเลี้ยงแพะขุนให้ได้น้ำหนักที่ลดลง ซึ่งเป็นประโยชน์แก่เกษตรกรโดยตรง

คำสำคัญ : โคโตโอลิโกแซกคลอไรท์, สมรรถภาพการเจริญเติบโต, โปรตีนรวม, คลอเรสเตอรอลรวม, แพะ

## Introduction

At present, there are growing interests in chitosan and chitooligosaccharide (COS) as functional food sources. Certain types of oligosaccharides have been used as prebiotics to improve animal performance, to enhance immune ability, and to affect gut microbial flora concentrations (White et al. 2002; Lemieux et al. 2003; Smiricky-Tjardes et al. 2003; Flemming et al. 2004). Those are being used for health products in human beings due to their cholesterol-lowering (Sugano et al. 1978, 1980; Maezaki et al. 1993) and anti-cancer actions (Suzuki et al. 1986; Tsukada et al. 1990). Chitooligosaccharide (COS) is an oligosaccharide that is easily obtained by chemical and enzymatic hydrolysis of poly-chitosan (Knaul et al. 1999). COS is a water-soluble chitosan, and it is produced via a natural enzymatic transformation of polymer chitosan into low molecular weight substance. COS has low molecular weight, good solubility, and low viscosity (Chae et al. 2005). Chitooligosaccharide has been shown to reduce the establishment of pathogens in the intestine (Shigehiro et al. 1990; Yalpani et al. 1992; Vishu Kumar et al. 2005) and improve immune function (Okamoto et al. 2003). It has also been shown to reduce the triglyceride level in obese diabetic mice (Hayashi and Ito, 2002). However, its role in regulating the blood lipid content is still controversial (Sugano et al. 1992; Ikeda et al. 1993). Chitooligosaccharide was also shown to have antifungal (Hirano and Nagao, 1989) and antimicrobial (Jeon et al. 2000) activities that improved gut health and thus increased nutrient digestibility and weight gain in broilers (Huang et al. 2005). In small ruminant animals such as goats, sheep, until now has limited data are available in terms of growth performance and physiological changes when COS is administered. Therefore, this study was conducted to determine the effects of feeding COS on growth performance, immunity and serum composition changes in order to make more weight gain for meat goat in Thailand.

## Materials and Methods

A total of 15 female goats (Native x Saanan; average initial body weight;  $28.5 \pm 0.5$  kg) were purchased from a standard private farm that approved by Department of Livestock Development (DLD). All goats were raised in wood-floored pen in an environmentally controlled house and had access to feed pangola grass and water ad libitum. The goats were employed to determine the effects of feeding commercial prebiotic (Nuclear C.O.S), a type of chitooligosaccharide (COS) on production traits (weight gain, ADG and FCR) immunity and serum composition (Total Cholesterol, Triglyceride, Total Protein) in goats. The Animal Welfare Committee of faculty of veterinary science, Mahidol University approved the animal care protocol used for this experiment.

### 1. Experimental Design and Diets

The goats were randomly allotted into 3 treatments with 5 replications each treatment. The CP 991-14 formula (B.P. Grower Goat: Chemical composition; Protein 14%, Fat 2.5%, Crude fiber 13%, Moisture 13%) was fed to all the goats. Average daily feed intake was 4.7% of body weight and fed pangola grass and water ad libitum. Diets were formulated to meet or exceed nutrient requirements as recommended by NRC (1998). The COS liquid was fed by oral route in the daily morning with 3 levels; 0 ppm (control) water 1 cc., 1 ppm: 1 cc. (LL) and 2 ppm: 2 cc (HL).

### 2. Sampling and Sample Processing Procedure

On day 0, 21, 42 and 63, the goats were weighed to determine average daily gain (ADG) and feed conversion rate (FCR). Blood samples 5 ml were collected from five goats per treatment on days 0, 21, 42 and 63 after feeding via venipuncture from the jugular vein, and they were put into tubes treated with EDTA as an anticoagulant 1 ml for complete blood count and for finding proportion of leukocyte and lymphocyte subpopulation and then centrifuged at  $3,000 \times g$  for 10 min to obtain serum. The serum samples were stored at  $-20^{\circ}\text{C}$  until needed for analysis.

### 3. Measurement of Serum Indices

The serum samples will keep as serum for checking blood chemistry - serum triglyceride, serum total protein, total cholesterol and HDL (High-density lipoprotein) cholesterol. The concentrations of total protein, triglyceride, total cholesterol, high-density lipoprotein (HDL) cholesterol in serum samples were analyzed by an automatic biochemical analyzer (RA-1000, Bayer Corp., Tarrytown, NY) using colorimetric methods, following the instructions of the manufacturer of the corresponding reagent kit (Zhongsheng Biochemical Co., Ltd., Beijing, China).

### 4. Statistical Analyses

A complete randomized design (CRD) was used in the experiment. Statistic analysis was carried out by comparing means using Duncan's multiple range

(Duncan, 1995) test, by General Linear Model (GLM) Procedure of SAS (1996) package program. A results value was less than 0.05 were considered significant.

## Results and Discussion

The ADG and FCR as affected by feeding COS (Nuclear C.O.S) were shown in Table 1. During growing periods, there were trends to improve ADG ( $p>0.05$ ), as COS (Nuclear C.O.S) levels were increased in the diets, even though it was not statistically significant. But FCR was reduced ( $p>0.05$ ) when COS (Nuclear C.O.S) level was high as compared to control level. The results showed that also improved gut health and thus increased nutrient digestibility and more weight gain in broilers (Huang et al. 2005).

**Table 1.** Growth performance of goats as affected by chitooligosaccharide (Nuclear C.O.S)

Parameters	Chitooligosaccharide <sup>1</sup>			
	Control	LL	HL	SE
ADFI, (g)	715.64	1129.94	855.86	115.57
ADG, (g)	16.67	30.96	22.23	16.09
FCR	42.93	36.40	38.50	32.26

<sup>1</sup>Add. level (Control: 0ppm, LL: 1ppm, HL: 2ppm).

The mean values of blood composition of the feed used in the experiment are shown in Table 2. The cholesterol and triglyceride did not differ between treatments ( $p>0.05$ ) but all mean values reduced after feeding COS (Nuclear C.O.S) supplementation. It has also been shown

to reduce the triglyceride level in obese diabetic mice (Hayashi and Ito, 2002) when we added the COS (Nuclear C.O.S) more. The total protein ( $p<0.05$ ) and HDL ( $p>0.05$ ) contents of serum were much higher than control when the goats intake more chitooligosaccharide level.

**Table 2.** Effects of chitooligosaccharide (Nuclear C.O.S) supplementation on blood chemistry of goats

Parameters	Chitooligosaccharide <sup>1</sup>			
	Control	LL	HL	SE
Total Cholesterol (mg/dl)	92.55 <sup>a</sup>	86.4 <sup>b</sup>	82.60 <sup>b</sup>	3.19
Triglyceride (mg/dl)	27.90	29.10	22.40	13.16
Total Protein (mg/dl)	5.87 <sup>b</sup>	6.40 <sup>a</sup>	6.11 <sup>ab</sup>	0.19
HDL (mg/dl)	41.27	48.08	41.18	4.70

<sup>1</sup> Add. level (Control: 0ppm, LL: 1ppm, HL: 2ppm).

<sup>ab</sup> Value with different superscripts of the same row are significantly differ ( $p < 0.05$ ).

The proportion of lymphocyte cells ( $p > 0.05$ ) were not significantly increased in chitooligosaccharide treated groups (Table. 3). However, the reduction of both types of Wbc and Rbc ( $p > 0.05$ ) in treated groups was not dependent on the treatment dose of chitooligosaccharide in feed. Because some types of oligosaccharides have

been used as prebiotics to improve immune ability, and to affect gut microbial flora concentrations (White et al. 2002; Lemieux et al. 2003; Smiricky-Tjardes et al. 2003; Flemming et al. 2004). So the effect of chitooligosaccharide on lymphoproliferation and phagocytic activity should be further investigated.

**Table 3.** Effect of chitooligosaccharide (Nuclear C.O.S) supplementation on changes of proportion of leucocytes subpopulations of goats

Parameters	Chitooligosaccharide <sup>1</sup>			
	Control	LL	HL	SE
Wbc ( $\times 10^6$ )	9.17	8.95	9.02	2.59
Rbc ( $\times 10^6$ )	5.31	5.51	5.09	0.29
Lymphocyte ( $\times 10^6$ )	65.05	66.35	66.40	3.52

<sup>1</sup> Add. level (Control: 0ppm, LL: 1ppm, HL: 2ppm).

## Conclusion

Effect of chitooligosaccharide supplementation on growth performance (FCR, ADG, ADFI) was higher than the control group although the number was not significantly. We found that the supplement with COS (Nuclear C.O.S) in water may help the weight gain of the goats more than a half time when compared the control group. The COS (Nuclear C.O.S) would be utilized more efficiently in goats fed diet containing COS (Nuclear C.O.S) than those fed control diets due to better condition of microbial populations in alimentary tracts. That things revealed that the total protein was improved ( $p < 0.05$ ) when COS (Nuclear C.O.S) level was higher than control. When we added the COS (Nuclear C.O.S) more, the total protein ( $p < 0.05$ ) and HDL ( $p > 0.05$ ) contents of serum were much higher than control because the goats intake more chitooligosaccharide level. The proportion of lymphocyte cells ( $p > 0.05$ ) was not significantly increased in chitooligosaccharide treated groups. At the present, it was not showed that the immunity system was improved in goats. A next study might be tested about the results on the immunity more.

## References

- Chae, S. Y., M. Jang, and J. Nah. (2005). Influence of molecular weight on oralabsorption of water soluble chitosans. *J. Control. Release* 102: 383-394.
- Duncan, D. B. (1955). Multiple range and multiple F test. *Biometrics* 11; 1-42.
- Flemming, J. S., J. R. S. Freitas, P. Fontoura, N. R. Montanhini, and J. S. Arruda. (2004). Use of mannono ligosaccharides in broiler feeding. *Brazil. J. Poul. Sci.* 6 : 159-161.
- Hayashi, K., and M. Ito. (2002). Antidiabetic action of low molecular weight chitosan in genetically obese diabetic KY-Ay mice. *Biol. Pharm. Bull.* 25 : 188-192.
- Hirano, S., and N. Nagao. (1989). Effects of chitosan, pectic acid, lysozyme and chitinase on the growth of several phytopathogens. *Agric. Biol. Chem* 53 : 3065-3066.
- Huang, R. L., Y. L. Yin, G. Y. Wu, T. J. Zhang, L. L. Li, M. X. Li, Z. R. Tang, J. Zhang, B. Wang, J. H. He, and X. Z. Nie. (2005). Effect of dietary oligochitosan supplementation on ileal nutrient digestibility and performance in broilers. *Poult. Sci.* 84: 1383-1388.
- Ikeda, I., M. Sugano, K. Yoshida, E. Sasaki, Y. Iwamoto, and K. Hatano. (1993). Effects of chitosan hydrolysates on lipid absorption and on serum and liver lipid concentration in rats. *J. Agric. Food Chem.* 41 : 431-435.
- Jeon, Y. J., F. Shahidi, and S. K. Kim. (2000). Preparation of chitin and chitosan oligomers and their applications in physiological functional foods. *Food Rev. Int.* 61 : 159-176.
- Knaul, J. Z., S. M. Hudson, and K. A. M. Creber. (1999). Cross-linking of chitosan fibers with dialdehydes: Proposal of a new reaction mechanism. *J. Poly. Sci. B: Polymer Physics* 37 : 1079-1094.
- Lemieux, F. M., L. L. Southern, and T. D. Bidner. (2003). Effect of mannan oligosaccharides on growth performance of weanling pigs. *J. Anim. Sci.* 81 : 2482-2487.
- Maezaki, Y., Tsuji, K., Nakagawa, Y., kawai, Y., Akimoto, M., Tsugita, T., Takekawa, W., Terada, A., hara, H. and Mitsuoka, T. (1993). Hypocholemic effect of chitosan in adult males. *Bioscience, Biotechnology and Biochemistry.* 57, 786-790.
- NRC. (1998). Nutrient requirement of swine (10<sup>th</sup> ed.). National Academy Press, Washington, D. C.
- Okamoto, Y., A. Inoue, K. Miyatake, K. Ogihara, Y. Shigemasa, and S. Minami. (2003). Effects of chitin/chitosan and their oligomers/monomers on migrations of macrophages. *Macromol. Biosci.* 3 : 587-590.

- SAS Institute. (1996). SAS User's Guide: Statistics. Version 7.0. SAS Institute, Cary, NC.
- Shigehiro, H., I. Chitoshi, and S. Haruyoshi. (1990). Chitosan as an ingredient for domestic animal feeds. *J. Agric. Food Chem.* 87 : 1214-1217.
- Smiricky-Tjardes, M. R., C. M. Grieshop, E. A. Flickinger, L. L. Bauer, and G. C. Fahey, Jr. (2003). Dietary galactooligosaccharides affect ileal and total-tract nutrient digestibility, ileal and fecal bacterial concentrations, and ileal fermentative characteristics of growing pigs. *J. Anim. Sci.* 81 : 2535-2545.
- Sugano, M., Fujikawa, T., Hiratsuji, Y., and Hasegawa, Y. (1978). Hypocholesterolemic effects of chitosan in cholesterol-fed rats. *Nutrition Reports International*, 18, 531-537.
- Sugano, M., Fujikawa, T., Hiratsuji, Y., Nakashima, K., Fukuda, N., and Hasegawa, Y. (1980). A novel use of chitosan as a hypocholesterolemic agent in rats. *American Journal Clinical Nutrition*. 33, 787-793.
- Sugano, M., K. Yoshida, M. Hashimoto, K. Enomoto, and S. Hirano. (1992). Hypocholesterolemic activity of partially hydrolyzed chitosans in rats. *Adv. Chitin Chitosan*. 84 : 472-478.
- Suzuki, K., Mikami, T., Oawa, Y., Tokoro, A., Suzuki, S. and Suzuki, M. (1986). Antitumor effect of hexa- N-acetylchitohexaose and chitohexaose. *carbohydr. Res.* 151: 403.
- Tsukada, K., Matsumoto, T., aizawa, K., Tokoro, a., Naruse, R., Suzuki, S. and Suzuki, M. (1990). Antimetastatic and growth-inhibitory effects of N-acetylchitohexaose in mice bearing lewis lung carcinoma. *Jpn. J. Cancer. Res.* 81: 259.
- Vishu Kumar, A. B., M. C. Varadaraj, L. R. Gowda, and R. N. Tharanathan. (2005). Characterization of chito-oligosaccharides prepared by chitosan analysis with the acid of papain and pronase, and their bactericidal action against *Bacillus cereus* and *Escherichia coli*. *Biochem. J.* 391:167-175.
- White, L. A., M. C. Newman, G. L. Cromwell, and M. D. Lindemann. (2002). Brewers dried yeast as a source of mannan oligosaccharides for weanling pigs. *J. Anim. Sci.* 80: 2619-2628.
- Yalpani, M., F. Johnson, and L. E. Robinson. (1992). Antimicrobial activity of some chitosan derivatives. *Adv. Chitin-Chitosan*. 5:543-548.