Effects of Chitosan-oligosaccharide on diarrhoea in Hanwoo calves

M.R. Alam[1], W.I. Kim[2], J.W. Kim[3], C.S. Na[4], N.S. Kim[2]

[1]Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh, Bangladesh
[2]College of Veterinary Medicine, Chonbuk National University, Jeonju, Korea
[3]College of Medical Science, Seonam University, Namwon, Korea
[4]College of Agriculture and Life Science, Chonbuk National University, Jeonju, Korea

ABSTRACT: The therapeutic effect of Chitosan-oligosaccharide (COS) on calf diarrhoea was investigated in Korean native (Hanwoo) calves ($n = 86$). The calves were divided into two groups; the untreated/control ($n = 40$) having routine diet only and the treated group ($n = 46$) receiving COS (50 ml/day) in addition to the routine diet for five days. Blood samples from each animal were collected before and after five days treatment and were subjected to complete blood count (CBC), chemistry screening (CS) and plasma protein assay (PPA). The *in vitro* efficacy of COS against the most common causal agents of calf diarrhoea (*Escherichia coli* and *Salmonella typhimurium*) was evaluated. Among the CBC parameters a significantly higher total red blood cell count (tRBC), haemoglobin (Hb) and packed cell volume (PCV) were noticed in the untreated group. In the CS parameters higher levels of blood urea nitrogen (BUN), creatinine and total bilirubin (TBL), and lower glucose were found in the untreated group. Significantly higher levels of albumin and α-globulin along with lower γ-globulin were noted in the untreated group. Among the treated group 41 (out of 46) calves recovered completely from diarrhoea and in the untreated group five calves (out of 40) spontaneously recovered after five days, revealing that the COS has a good therapeutic effect on diarrhoea in calves. The COS was found to effectively inhibit bacterial growth and pathogenicity up to 1:64 and 1:256 dilutions in the case of *S. typhimurium* and *E. coli* respectively. The results of this study revealed that the levels of different parameters of CBC (tRBC, Hb, PCV), CS (BUN, creatinine, TBL) and PPA (albumin, α-globulin, γ-globulin) profiles can reflect the severity of diarrhoea and dehydration and that COS can be successfully used for the clinical management of diarrhoea in Hanwoo calves.

Keywords: Chitosan; complete blood count; chemistry screening; plasma proteins; diarrhoea; calf

Diarrhoea is an important problem in young domestic animals although its etiology is not well understood since several agents may be involved concurrently (Munoz et al. 1996). Diarrhoea in calves can be caused by a variety of pathogens including bacteria, viruses, protozoa and intestinal parasites. Among bacteria, enterotoxigenic *Escherichia coli* and *Salmonella* are known to be the most common and economically important agents (Ganaba et al. 1995; Acha et al. 2004). Diarrhoea in neonatal calves is a major source of economic loss to the cattle industry (Walker et al. 1998). It is the leading cause of death in dairy heifer and beef calves aged less than four months. Financial losses occur not only from calf mortality, but also from the cost of medication and labour needed to treat and care for the sick calves (Walker et al. 1998). The conventional treatment of diarrhoea (gut active antibacterials and astringents) usually requires a longer period of time or sometimes fails to result in the recovery of the affected animals. Therefore, it is of great importance to discover alternative thera-

peutic agents for effective treatment of the disease in order to combat the significant economic losses.

Chitosan, a natural polysaccharide biopolymer, has received considerable attention as a functional, nontoxic and biodegradable biopolymer for diverse applications, especially in pharmaceutics, food and cosmetics (Dodane and Vilivalam 1998; Shahidi et al. 1999; Jeon et al. 2000; Kumar et al. 2004). Chemically, it is a high molecular weight linear polycationic heteropolysaccharide consisting of two monosaccharides, *N*-acetyl-D-glucosamine and D-glucosamine, linked together by β-(1→4) glycosidic bonds (Liu et al. 2006). In the medical field, chitosan has been developed not only as artificial skin, absorbable surgical suture, and a wound healing accelerator, but also as a new physiological material due to its antitumour, immune-enhancing, antimicrobial and hypocholesterolemic properties (Zhang et al. 2010).

Recently, chitosan has also attracted considerable attention in veterinary applications, as a wound healing agent, antimicrobial agent, bandage material, skin grafting template, haemostatic agent, space-filling implant and drug delivery vehicle (Senel and McClure 2004).

The significance of determining haematological and biochemical indices of domestic animals has been well documented (Oduye and Fasanmi 1971). Complete blood count (CBC), chemistry screening (CS) and plasma protein assay (PPA) can be important and powerful diagnostic tools as a component of minimum databases. It can be used to monitor the response to therapy, to gage the severity of an illness or as a starting point for formulating a list of differential diagnosis (Barger 2003). It is well known that variables such as breed, stage of growth, age, reproduction status and stage of lactation have an influence on many blood parameters (Doornenbal et al. 1988). As production is closely related to the health and nutrition status of all animals, haematological and plasma biochemical evaluation is considered to be a basic aid in this determination. Haematological and biochemical values are also used for indicating stress and welfare. There are a number of parameters that are known or proposed to be useful in measuring calf predisposition to morbidity or mortality; some of them are the total protein (TP), albumin, total red blood cell count (tRBC), haemoglobin (HB) and packed cell volume (PCV) (Pare et al. 1993). However, little is known about how these variables affect the risk of diarrhoea or reflect the

severity of the disease. The extent of knowledge on the changes in these variables associated with diarrhoea would be useful in developing preventive measures and assessing the prognosis of individual cases (Pare et al. 1993). To the best of our knowledge there is a lack of published reports on the effect of Chitosan-oligosaccharide (COS) on diarrhoea. Therefore, this study evaluated the therapeutic efficacy of COS on diarrhoea as well as the changes in the haematological, biochemical and plasma protein profile in diarrhoea-affected Korean native (Hanwoo) calves.

MATERIAL AND METHODS

Experimental animals

This study was conducted during the period of March 2010 to February 2011, Jeollabukdo Province, Republic of Korea. During this period a total of 86 Hanwoo calves, one to six months of age, raised using ordinary husbandry were found to be clinically affected with diarrhoea. The affected calves were selected on the basis of farm records and physical examination at the early stage of the disease. The calves were divided into two groups; the control (*n* = 40) having routine diet only and the treated group (*n* = 46) receiving COS (50 ml/day) in addition to the routine diet for five days.

Physical examination of the diarrhoea-affected calves

The diarrhoea-affected calves were inspected for the classic signs of dehydration: sunken eyes, dry mouth and nose, weight loss, fast or very slow pulse, cold ears and/or cold legs. Then, the tenting test was performed to evaluate the state of dehydration. The loose fold skin on the neck was firmly pinched and checked to see how long the skin remained tented and the dehydration status was recorded as normal (skin remained tented less than two seconds) mild (skin remained tented for two to three seconds), moderate (skin remained tented for three to six seconds) and severe (skin remained tented for more than six seconds). The calves having mild and moderate dehydration received routine diet and COS orally and those having severe dehydration in addition to the routine diet and COS received intravenous fluid therapy for

quick rehydration. The recently defecated faeces of the affected calves were grossly examined for consistency (normal, loose, very loose, and runny), odour and colour.

Preparation of the Chitosan-oligosaccharide (COS)

COS was prepared by enzymatic hydrolysis from low molecular chitosan showing more than 98% of deacetylation and lower than 10 cps viscosity according to the method reported by Jeon et al. (2001). This involves the continuous hydrolysis of chitosan in an UF membrane reactor system connected to an immobilised enzyme column reactor in which chitosanase from *Bacillus* sp. was adsorbed on chitin as a carrier for immobilisation. Three different ultrafiltration (UF) membranes having molecular weight cut-offs (MCWO) of 10, 5, and 1 kDa were used in the system. One percent chitosan solution (pH 5.5) was passed through the packed column reactor containing the immobilised enzyme at an output flow rate of 5 ml/min to obtain a partially hydrolysed chitosan (PHC) solution. Subsequently, the PHC was continuously added to the UF membrane reactor system for the enzymatic conversion of PHC to COS. Three kinds of COS were prepared using this system: high molecular weight COS (passed through the 10 kDa MWCO membrane but not the 5 kDa membrane); medium molecular weight COS (passed through the 5 kDa membrane but not the 1 kDa membrane) and low molecular weight COS (passed through the 1 kDa membrane). The COS was composed of 7.8% monomer, 0.8% dimer, 13.1% trimer, 18.5% tetramer, 21.3% pentamer, 30.3% hexamer, and 8.2% more than heptamer.

Analysis of blood samples

Five ml of blood samples from the calves of both treated and untreated groups were collected by jugular venepuncture prior to the treatment and after five days of oral administration of COS. Immediately after collection 2 ml of the samples were transferred to sterile screw-capped tubes containing EDTA for haematology and the remaining 3 ml to tubes containing lithium heparin for biochemistry. Blood samples were transported to the laboratory within one hour in a thermoflask with ice and samples were examined immediately. The heparinised samples

were centrifuged at 1500g for 15 min, after which the supernatant plasma was collected, divided into two halves, transferred to Eppendorf tubes and stored at −80 °C until analysed by CS and PPA.

The blood samples were subjected to CBC which includes total white blood cell count (tWBC), total red blood cell count (tRBC), haemoglobin (Hb), packed cell volume (PCV), platelet count, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC); and CS which includes alkaline phosphatase (AP), aspartate aminotransferase (AST), blood urea nitrogen (BUN), creatinine, glucose, lactate dehydrogenase (LDH), total bilirubin (TBL), total protein (TP), albumin, albumin/globulin (A/G) ratio and to immunoassay for various fractions of globulins (alpha, beta and gamma).

The CBC was analysed using an automatic haematology analyser (Scil Vet abc, Scil Animal Care Company, USA). Briefly, the EDTA-blood sample tubes were placed on a roller mixer and rolled at 33 rpm and 16 mm amplitude for proper mixing. The sample identity was entered in the analyser and once the sample needle was down it was put into the tube containing the blood samples. Once the start button was pressed, 20 ml of the blood sample were automatically taken in by the sample needle. The results were available on the LCD after 90 s and were received in printed form as well.

For CS analysis 90 ml of plasma were drawn from the Eppendorf tubes using an automatic pipettor/dilutor. The plasma was then dispensed into a test rotor (VET-16 Veterinary Test Rotor®, Hemagen Diagnostics, Inc., USA) and the rotor cap was snapped into place. The test rotor was loaded into an automatic chemistry analyser (Hemagen Analyst®, Hemagen Diagnostics, Inc., USA) and a complete result was received in printed form in about 10 min. For immunoassay the plasma samples were sent to the Samkwang Medical Laboratory, Seoul, Korea. The immunoassay for various fractions of globulins (alpha, beta and gamma) was done by a specialist of the laboratory using specific kits for the assay.

In vitro effect of Chitosan-oligosaccharide on *Escherichia coli* and *Salmonella typhimurium*

The antibacterial effect of COS was tested against *Escherichia coli* and *Salmonella typhimurium*. COS

was used as a two-fold serial dilution with LB Broth (USB corporation, Cleveland, OH, USA) in 24-well flat bottom cell culture plates (BD FALCON 24-well, Franklin lakes, NJ, USA) up to nine time points (1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256 and 1:512). After making dilutions in 24-well plates, 200 CFU/ml (Colony-forming unit per milliliter) of both bacteria were inoculated into each well containing diluted COS. After that, the plate was incubated in a shaking incubator (JSR, Labtech) at 37 °C temperature for the next 16 h to allow bacterial growth. After 16 h of incubation, bacterial growth was observed and the end point of growth was determined. Bacterial subculture (plated) was performed on LB agar plates from each dilution of COS containing bacterial growth for confirmation and the CFU/ml was determined to establish the number of viable bacteria (bacterial load) as well as magnitude of infectivity for each respective dilution of COS.

Statistical analysis

The data were analysed using the analyses of variance (ANOVA) technique and Student's *t*-test.

RESULTS

Therapeutic effects of Chitosan-oligosaccharide on diarrhoea

In the untreated group (*n* = 40) initially 31 calves were found to have loose and nine calves very loose faeces. After five days spontaneous recovery was observed in five calves while the disease condition deteriorated in others (Loose faeces-five, Very loose-32 and Runny in three calves). In the COS group (*n* = 46) initially 36 calves with loose and 10 calves with very loose faeces were found. After five days of treatment with COS 41 calves completely recovered while diarrhoea persisted in the other five (Loose-two, Very loose-two and Runny faeces-one). There was a significantly higher (*P* < 0.05) rate of recovery from diarrhoea (41 out of 46 calves, 89%) observed in the COS-treated group in comparison to that of the untreated group (five out of 40 calves, 12%).

In the untreated group (*n* = 40), 28 calves were found to be normally hydrated (having no dehydration) and 12 calves had mild dehydration ac-

Table 1. Therapeutic effects of Chitosan-oligosaccharide on diarrhoea in Hanwoo calves

Parameters	Routine diet group (*n* = 40)		Treated group (*n* = 46)	
	control	5 days after normal diet	control	5 days after COS treatment
Faeces				
Normal	–	5	–	41
Loose	31	5	36	2
Very loose	9	32	10	2
Runny	–	3	–	1
Dehydration (skin tenting test)				
Normal (< 2 s)	28	5	31	40
Mild (2–3 s)	12	11	15	1
Moderate (3–6 s)	–	20	–	3
Severe (> 6 s)	–	4	–	2

cording to the skin tenting test. After five days the dehydration score in the calves was found to be increased (Normal-five, Mild-eleven, Moderate-twenty Severe-four). In the COS group (*n* = 46) initially 31 calves were found to be normally hydrated (having no dehydration) while 12 calves had mild dehydration according to the skin tenting test. After five days of treatment with COS the dehydration score in the calves was found to be decreased (Normal-forty and Mild-one). However, in five calves the dehydration deteriorated (Moderate-three and Severe-one). The pre-treatment and post-treatment faeces and dehydration scores are shown in Table 1.

The complete blood count (CBC)

In this study the CBC values of all the groups were found to be within the normal reference range (Jian, 1986). A decrease in the tWBC value (6.00 ± 1.95 × 10^3/ml) was found after five days in the untreated control group in comparison to that of the pre-treatment control (6.94 ± 3.45 × 10^3/ml) and COS-treated groups (6.98 ± 2.70 × 10^3/ml). However, this difference in values was not statistically significant. Significantly (*P* < 0.05) higher tRBC (9.22 ± 1.45 × 10^6/ml), HB (11.76 ± 2.13 g/dl) and PCV (45.85 ± 6.86%) were observed after five days in the untreated control group in comparison to that of the pre-treatment control (tWBC 6.54 ± 3.45 × 10^3/ml,

Table 2. The complete blood count (CBC) values (mean ± SD) in the diarrhoea affected Hanwoo calves ($n = 86$)

Parameters	Pretreatment control ($n = 86$)	Routine diet group ($n = 0$)	Treated group ($n = 46$)	Reference range
tWBC (10^3/ml)	6.94 ± 3.45	6.00 ± 1.95	6.98 ± 2.70	4.00–12.00
tRBC (10^6/ml)	7.88 ± 1.46	9.22 ± 1.45*	8.35 ± 1.25	5.00–10.00
Hb (g/dl)	8.26 ± 1.65	11.76 ± 2.13*	9.15 ± 1.32	8.00–15.00
PCV (%)	35.56 ± 5.65	45.85 ± 6.86*	38.75 ± 3.75	24.00–46.00
Platelet (10^3/ml)	607.92 ± 205.15	683.15 ± 235.22	657.12 ± 215.67	200–800
MCV (fl)	45.12 ± 3.32	49.72 ± 4.85	46.40 ± 4.46	40.00–60.00
MCH (pg)	10.48 ± 0.15	12.75 ± 0.18	10.95 ± 0.12	11.00–17.00
MCHC (g/dl)	23.22 ± 1.44	25.64 ± 1.32	23.61 ± 0.85	28.20–38.00

SD = standard deviation, tWBC = total white blood cell count, tRBC = total red blood cell count, Hb = haemoglobin, PCV = packed cell volume, MCV = mean corpuscular volume, MCH = mean corpuscular haemoglobin, MCHC = mean corpuscular haemoglobin concentration
*statistically significant $P < 0.05$ or less

tRBC 7.88 ± 1.46 × 10^6/ml, HB 8.26 ± 1.65 g/dl, PCV 35.56 ± 5.65%) and COS-treated group (tWBC 6.80 ± 2.70 × 10^3/ml, tRBC 8.35 ± 1.25 × 10^6/ml, HB 9.15 ± 1.32 g/dl, PCV 38.75 ± 3.75%). There were no significant differences in the values of platelets, MCV, MCH and MCHC among the groups during the experiment. The CBC values in the diarrhoea-affected Hanwoo calves ($n = 86$) is presented in Table 2.

Chemistry screening (CS) and plasma protein assay (PPA)

The values of the AP, AST and LDH were within the reference range in all the groups (Jenkins et al. 1982). There were no significant differences in these parameters among the groups during the

experiment. Significantly higher ($P < 0.05$) BUN (25.32 ± 4.45 g/dl), creatinine (1.26 ± 0.16 g/dl) and TBL (1.46 ± 0.17 g/dl) were observed in the untreated group in comparison to the COS-treated group (BUN 21.65 ± 3.45 g/dl, creatinine 1.16 ± 0.26 g/dl, TBL 1.15 ± 0.28 g/dl) and pre-treatment control values (BUN 21.28 ± 2.44 g/dl, creatinine 1.15 ± 0.27 g/dl, TBL 1.12 ± 0.25 g/dl). Significantly lower ($P < 0.05$) glucose levels (72.17 ± 18.92 g/dl) were noted in the untreated group in comparison to the COS-treated group (114.22 ± 14.25 g/dl) and pre-treatment control value (99.54 ± 4.65 g/dl).

There was no significant variation in the total protein (TP) values in the untreated group (6.85 ± 0.37 g/dl) in comparison to the COS-treated group (7.08 ± 0.40 g/dl) and pre-treatment control value (6.90 ± 0.67 g/dl). Significantly higher ($P < 0.05$) albumin was observed in the untreated group

Table 3. Blood chemistry screening (CS) values (mean ± SD) in the diarrhoea affected Hanwoo calves ($n = 86$)

Parameters	Pretreatment control ($n = 86$)	Routine diet group ($n = 40$)	Treated group ($n = 46$)	Reference range
AP (IU/l)	215.50 ± 45.50	225.40 ± 22.64	218.65 ± 22.15	66.00–220.00
AST (IU/l)	26.58 ± 0.80	22.22 ± 4.63	24.00 ± 5.26	7.00–38.00
BUN (mg/dl)	21.28 ± 2.44	25.32 ± 4.45*	21.65 ± 3.45	6.00–23.00
Creatinine (mg/dl)	1.15 ± .27	1.26 ± 0.16*	1.16 ± 0.26	0.40–1.40
Glucose (mg/dl)	99.54 ± 4.65	72.17 ± 18.92*	114.22 ± 14.25	75.00–128.00
LDH (IU/l)	260.35 ± 42.22	233.64 ± 32.62	254.43 ± 36.28	105.00–260.00
TBL (mg/dl)	1.12 ± 0.25	1.46 ± 0.17*	1.15 ± 0.28	0.20–1.40

SD = standard deviation, AP = alkaline phosphatase, AST = aspartate aminotransferase, BUN = blood urea nitrogen, LDH = lactate dehydrogenase, TBL = total bilirubin
*statistically significant $P < 0.05$ or less

Table 4. The concentration (mean ± SD) of total protein, albumin and various fractions of globulin in the diarrhoea affected Hanwoo calves (*n* = 86)

Parameters	Pretreatment control (*n* = 86)	Routine diet group (*n* = 40)	Treated group (*n* = 46)
TP g/(dl)	6.90 ± 0.67	6.85 ± 0.37	7.08 ± 0.40
A/G	0.94 ± 0.13	1.14 ± 0.17	0.87 ± 0.10
Albumin (%)	48.45 ± 3.60	53.23 ± 3.87*	46.53 ± 3.15
Globulin (%)	51.55 ± 2.75	46.77 ± 2.29	53.47 ± 2.53
α-Globulin (%)	10.20 ± 1.27	13.47 ± 1.24*	12.00 ± 1.78
β-Globulin (%)	11.55 ± 1.06	11.12 ± 1.61	11.07 ± 1.85
γ-Globulin (%)	29.8 ± 5.93	22.15 ± 4.02*	30.40 ± 3.98

SD = standard deviation. TP = total protein, A/G = albumin/globulin
*statistically significant *P* < 0.05 or less

(53.23 ± 3.87%) in comparison to the COS-treated group (46.53 ± 3.15%) and pre-treatment control value (48.45 ± 3.60%). Among the globulins, significantly higher levels of (*P* < 0.05) α-globulin (13.47 ± 1.24%) and lower γ-globulin (22.15 ± 4.02%) were measured in the untreated group in comparison to the COS-treated group (α-globulin 12.00 ± 1.78%, γ-globulin 30.40 ± 3.98%) and pre-treatment control value (α-globulin 10.20 ± 1.27%, γ-globulin 29.8 ± 5.93%). The CS and PPA values of the diarrhoea-affected Hanwoo calves are presented in Table 3 and 4.

In vitro effect of Chitosan-oligosaccharide on *Escherichia coli* and *Salmonella typhimurium*

Bacterial growth was observed from 1:32 (*E. coli*) and 1:128 (*S. typhimurium*) dilutions but they was

Figure1. Effect of chitosan-oligosaccharide on *Escherichia coli* and *Salmonella typhimurium*

no pathogenicity up to 1:64 (*E. coli*) and 1:256 (*S. typhimurium*) dilutions of Chitosan-oligosaccharide. The *in vitro* effect of COS is presented in Figure 1.

DISCUSSION

Diarrhoea in calves can be caused by a variety of pathogens including bacteria, viruses, protozoa and intestinal parasites. Among bacteria, enterotoxigenic *Escherichia coli* (ETEC) and *Salmonella* are known to be the most common and economically important agents (Acha et al. 2004). In our study the selected farms had a known prevalence of *E. coli* and *Salmonella*.

Chitosan is a partially deacetylated polymer obtained from the alkaline deacetylation of Chitin, which is the most abundant biopolymer in nature after cellulose. It is a polysaccharide widely distributed in nature as the principal component of exoskeletons of crustaceans and insects as well as of cell walls of some bacteria and fungi (Senel and McClure, 2004). Because of its unique polycationic nature, chitosan has been found to possess antifungal activity (Roller and Covill 1999) antibacterial activity (Helander et al. 2001; Liu et al. 2006) and antitumor activity (Qin et al. 2001). Recently, the conversion of chitin and chitosan to oligosaccharides has attracted interest because oligosaccharides are not only water-soluble but also possess versatile functional properties such as antitumor activity, immuno-enhancing effects, enhancement of protective effects against infection with some pathogens, antifungal activity, and antimicrobial activity. With respect to antibacterial activity, chitosan is superior to chitin since it possesses many
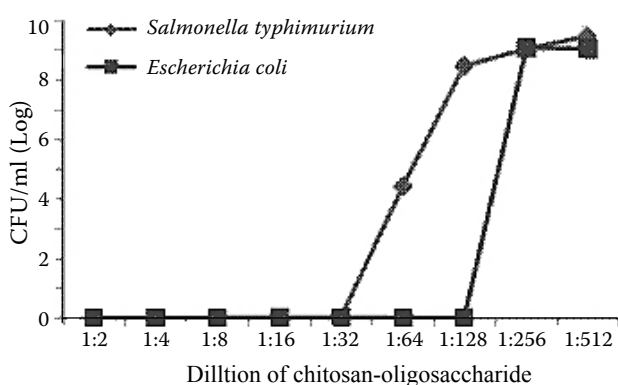
polycationic amines which interact with the negatively charged residues of macromolecules at the cell surface of bacteria and in this manner inhibit their growth (Jeon et al. 2001). In our study we also used Chitosan-oligosaccharide (COS).

We observed that COS exerted an excellent therapeutic effect on diarrhoea in Hanwoo calves. Among 46 medicated calves 41 (89%) recovered from diarrhoea and the hydration status was also found to be improved after five days treatment with COS. This finding cannot be compared and contrasted with the literature because of the lack of similar reports. However, the therapeutic effect of COS is thought to be due to its antibacterial effects against enterotoxigenic *E. coli* and *Salmonella*. Its therapeutic effects also correlate with its *in vitro* effects against *E. coli* and *S. typhimurium*. The *in vitro* effect of COS against *E. coli* and *S. typhimurium* is in agreement with previous reports (Helander et al. 2001; Je and Kim 2006). The actual mechanism of antimicrobial activity of chitosan and its derivatives is not yet fully understood but has been variously suggested to involve cell lysis and breakdown of the cytoplasmic membrane barrier. Additionally, the trace metal cations selectively chelated by the chitosan could be necessary for microorganismal growth (Helander et al. 2001; Chung et al. 2004). To kill gram-negative bacteria, a cationic chitosan must interact with both bacterial cell envelope membranes. Chitosan disrupts the barrier properties of the outer membrane of gram-negative bacteria (Helander et al. 2001). It has been also reported that in dilute acid solutions, the positive charges of chitosan interferes with the negatively charged residues of macromolecules at the bacterial cell surface, presumably by competing with Ca^+ for electronegative sites on the membrane without conferring dimensional stability, rendering the membrane leaky (Begin and Calsteren 1999; Senel and McClure 2004). However, little is known regarding the antimicrobial activity of chitin derivatives (Je and Kim 2006).

The importance of haematological and biochemical indices in animal husbandry is well acknowledged (Oduye and Fasanmi 1971). The physiological equilibrium of the body is maintained mainly by the blood, but many physiological conditions may alter this equilibrium. Changes in haematological constituents are important indicators of the physiological or pathological state of the animal. Blood examination is also performed for screening procedures which assess the general health of animals (Jian

1986). In this study, among the CBC parameters, relatively lower tWBC and significantly higher tRBC, HB and PCV were observed with the progression of diarrhoea along with dehydration scores in the untreated control group in comparison to that of the pre-treatment control values and COS-treated group. This finding is in agreement with the previous reports of changes in these parameters in diarrhoea-affected calves (Pare et al. 1993; Santos et al. 2002). The higher levels of tRBC, Hb and PCV in the untreated animals is thought to be due to haemoconcentration with the progression of dehydration caused by a decrease in fluid intake and excessive fluid loss due to diarrhoea (Pare et al. 1993; Santos et al. 2002). The mild leukopenia observed in the untreated calves is in agreement with the report of Santos et al. 2002. The actual mechanism behind this is not clear. However, it is thought to be due to infiltration of a massive number of neutrophils into the intestinal mucosa from the blood stream which also might be correlated with the increased intestinal fluid loss during disease progression.

The significantly higher levels of BUN, creatinine and TBL, and lower glucose levels observed in the untreated group with the progression of the disease in comparison to the COS treated group and pre-treatment control values are in agreement with the report of these parameters in diarrhoea-affected calves (Santos et al. 2002). In untreated animals, this progressive dehydration resulted in inadequate renal perfusion which could explain the increase in BUN and creatinine levels. The increase in the TBL might be due to the diminished uptake of unconjugated bilirubin from plasma by hepatocytes and decreased reflux of conjugate bilirubin from hepatocytes which result from dehydration and a lack of food intake (Santos et al. 2002). The lower glucose levels were also due to the increased intestinal fluid loss, decreased absorption of nutrients and lack of food intake during the disease process.

Significantly higher levels of albumin were found in the untreated group in comparison to the COS-treated group and pre-treatment control value. This finding is in agreement with earlier reports (Thorton et al. 1972; Santos et al. 2002). Among the globulins, significantly higher levels of α-globulin and lower γ-globulin were noted in the untreated group in comparison to the COS-treated group and pre-treatment control value which is in agreement with a previous report (Thorton et al. 1972). Though the catabolic rates of the serum proteins were not directly determined, the significant higher

albumin, α-globulin and lower γ-globulin concentrations with the progression of diarrhoea and dehydration in the untreated group is noteworthy. These findings, together with the unchanged TP over the course of the clinical progression of the disease, suggest that the concentrations of albumin and α-globulin related to water loss and increased rates of γ-globulin catabolism.

The results of this study revealed that the levels of different haematological (increased tRBC, Hb and PCV), biochemical (increased BUN, creatinine and TBL) and plasma protein parameters (increased albumin, α-globulin and decreased γ-globulin) can reflect the severity of diarrhoea and dehydration in affected animals. We also conclude that the Chitosan-oligosaccharide can be successfully used for the clinical management of diarrhoea in Hanwoo calves.

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Corresponding Author:

Nam Soo Kim, Chonbuk National University, College of Veterinary Medicine, Department of Surgery, Jeonju 561-756, Korea

Tel. + 82 10 9601 2801, Fax +82 63 270 3780, E-mail: namsoo@jbnu.ac.kr