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Effects of Non-starch Polysaccharide-degrading Enzymes on Nutrient Digestibility, Growth Performance and Blood Profiles of Growing Pigs Fed a Diet Based on Corn and Soybean Meal

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ABSTRACT : Two experiments with growing pigs were conducted to investigate the effects of two distinct multienzyme preparations on nutrient digestibility, growth performance and blood profiles. In Exp. 1, a total of 96 pigs (29.7±0.69 kg) were utilized in a 42-day performance and digestibility trial using four dietary treatments: CON (control diet), ENDO (control+0.10% Endopower), NSPase1 (control+0.10% NSPase) and NSPase2 (control+0.20% NSPase). Endopower was a commercial multienzyme preparation which contained α-galactosidase, galactomannase, xylanase and β-glucanase. NSPase mainly contained α-1,6-β-galactosidase, β-1,4-mannanase and β-1,4-mannosidase. There were six replication pens per treatment with four pigs per pen. Pigs fed NSPase1 diet had a higher ADG ($p<0.05$) and G:F ($p<0.05$) than those fed the control diet. There were no significant differences in growth performance among the multienzyme treatments ($p>0.05$). Compared with CON, apparent digestibility of DM was increased ($p<0.05$) by ENDO treatment. N digestibility was improved ($p<0.05$) in response to multienzyme treatments during the experimental period. Blood urea nitrogen (BUN) was higher ($p<0.05$) in ENDO treatment than in CON and NSPase1 treatments at the end of the experiment, while the glucose level improved ($p<0.05$) due to ENDO and NSPase2 treatments. In Exp. 2, four ileal-cannulated, growing barrows (20.17±1.31 kg) were housed in individual metabolism crates and randomly assigned to 1of 4 treatments (same as Exp. 1) within a 4×4 Latin square design. Enzyme supplementations improved the majority of apparent ileal amino acid digestibilities ($p<0.05$). It is concluded that the supplementation of NSPase1 improved growth performance as well as N digestibility and partially improved apparent ileal amino acid digestibility in growing pigs fed a diet based on corn and soybean meal. (**Key Words :** Enzymes, Pigs, Performance, Digestibility, Blood Profiles)

INTRODUCTION

Soluble nonstarch polysaccharides (NSP) have anti-nutritional effect and cannot be degraded by pigs because pigs cannot excrete NSP enzymes. Therefore, there has been considerable interest in applying exogenous non-starch polysaccharides degrading enzyme preparations for potential benefits. The effects of exogenous enzymes on growth performance and nutrient digestibility may be influenced by enzyme preparations, the physiological status of the animal and feed ingredient. Numerous studies show the most likely application would be in weanling pigs (Omogbenigun et al., 2004; Shim et al., 2004). However, exogenous enzymes studies with older pigs appear less effective because older pigs are more able to digest fiber than younger pigs.

For dietary composition as enzyme substrates, a lot of investigation with fiber-degrading enzymes has been widely carried on wheat, barley, rye, corn or sorghum based diets (Bedford and Partridge, 2001). Owing to decreased fiber content and high nutrient digestibility, it has been more challenging to obtain beneficial effects for corn and soybean meal (SBM) diets through the exogenous enzymes supplementation. It is well known that corn and SBM based diets are widely used in most parts of the world. According to CVB (1998), corn and SBM contain 10 and 20% NSP, respectively. There may be potential benefits for a response to the use of exogenous enzymes. Besides, Graham et al. (1988) suggested that a combination of different enzyme activities is required for degradation of complex NSP and improved nutrient utilization.

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Liener (1994) reviewed the antinutritional compounds in soybeans, most of which can be eliminated during the heat processing of soybeans into soybean meal. Nevertheless, α-galactomannans and α-1,6-galactosides still exist after soybean meal processing (Hartwig et al., 1997; Rackis, 1981). Trugo et al. (1995) reported that the amount of α-1,6-galactosides (raffinose, 1.0% and stachyose, 4.6%) and β-galactomannans (1.2%) are relatively high in soybean meals which cannot be digested by pigs because they lack enzymes targeting α-1,6-galactosyl bonds and β-1,4-mannosyl bonds (Veum and Odle, 2001). As is known to all, these antinutritional compounds may result in the decrease in nutrient digestibility and growth rate.

Both enzymes used in this study are targeted to degrade the above antinutritional compounds. Therefore, the objective of this experiment was to evaluate the effects of two kinds of enzyme cocktail on nutrient digestibility, growth performance and blood profiles of growing pigs fed diets based on corn and SBM.

MATERIALS AND METHODS

Enzyme preparation

Endopower preparation contains 7 unit/g α-galactosidase activity, 22 unit/g galactomannanase activity, 300 unit/g xylanase activity and 220 unit/g β-glucanase activity. NSPase preparation was guaranteed to have 7 unit/g of α-1,6-β-galactosidase and 22 unit/g of β-1,4-mannanase. One unit of α-galactosidase is defined as the amount of enzyme that liberates 0.1 μmol nitro phenol from 2 mmol of pNPG (p-nitrophenyl-alpha-dgalactoside) per at 30°C and pH 4.0. One unit of galactomannase is defined as the amount of enzyme that liberates 0.1 μmol total reducing sugars/min from 0.5% galactomannan per at 30°C and pH 4.0. One unit of xylanase is defined as the amount of enzyme that liberates 1 mg total reducing sugar/10 min. from 0.5% xylan at 30°C and pH 4.0. One unit of β-glucanase is defined as the amount of enzyme that liberates 1 mg of total reducing sugar per 10 min. from 0.4% β-glucan at 30°C and pH 4.0.

NSPase preparation used for this study was comprised of 40% dehydrated fermentation products from Aspergillus niger (PRL 2351) and Aspergillus oryzae (ATCC 66222) by weight and 60% dehydrated barley malt sprouts. The major active enzymes of the preparation were α-1,6-β-galactosidase, β-1,4-mannanase and β-1,4-mannosidase, although it also contains several minor residual enzymes including β-1,4-glucanase, β-1,4-glucosidase, cellobiase, xylosidase, arabinosidase, and amiloglucosidase. The preparation was guaranteed to have 7 unit/g of α-1,6-β-galactosidase (one unit is the enzyme activity required to hydrolyze 1 μmol of p-nitrophenyl-β-D-galactopyranoside within 1 min at 30°C and pH 4.0) and 22 unit/g of β-1,4-mannanase (one unit is the enzyme activity required to release 1 mg of total reducing sugars-glucose equivalent within 1 min at 30°C and pH 4.0).

Exp. 1

Experimental design, animals and diets : The experimental protocol used in this study was approved by the Animal Care and Use Committee of Dankook University. A total of 96 crossbred pigs [(Landrace×Yorkshire)×Duroc] with an average initial BW of 29.7±0.69 kg were selected for this 6-week growth assay. Pigs were randomly allocated to 1 of 4 treatments according to their BW, sex and litter with 6 replicates per treatment and 4 pigs per pen (2 females and 2 males). The dietary treatments were as follows: CON (control diet), ENDO (control+0.10% Endopower), NSPase1 (control+0.10% NSPase) and NSPase2 (control+0.20% NSPase). All the diets were formulated to meet NRC (1998) recommendation (Table 1). Pigs were housed in an environmentally controlled, slatted-floor facility in 24 adjacent pens (1.8×1.8 m) and were allowed *ad libitum* access to feed and water. The target room temperature and humidity were maintained at 24°C and 60%, respectively.

Sampling and measurement : For the growth assay, the

Table 1. Diet composition (as-fed basis)

Ingredients (%)	Basal diet
Corn	54.87
Soybean meal	31.03
Rapeseed meal	1.60
Rice bran	1.50
Tallow	4.35
Molasses	3.10
Dicalcium phosphate	1.18
Limestone	0.79
Salt	0.20
L-lysine (74%)	0.34
Vitamin premix[1]	0.20
Trace mineral premix[2]	0.10
Calculated composition	
DE (kcal/kg)	3,400
Crude protein (%)	17.00
Lysine (%)	0.98
Calcium (%)	0.75
Phosphorus (%)	0.62

[1] Provided per kg of complete diet: 6,500 IU vitamin A, 950 IU vitamin D_3, 27 IU vitamin E, 2.0 mg vitamin K_3, 3.6 mg vitamin B_2, 1.3 mg vitamin B_6, 15 mg pantothenic acid, 26.0 mg niacin and 0.03 mg biotin.

[2] Provided per kg of complete diet: 50 mg Mn (as manganese oxide), 70 mg Zn (as zinc oxide), 54 mg Cu (as copper sulfate), 0.5 mg I (as calcium iodate), 0.5 mg Co and 0.25 mg Se.

individual pig weight and feed consumption were recorded at the onset and the termination of the experiment for the determination of ADG, ADFI and G:F. Chromic oxide (0.20%) was added as an inert indicator to calculate the apparent digestibility of DM, N and energy. After the pigs were fed the diets containing the indicator for 7 days, fresh fecal grab samples were obtained from each pen at the end of the experiment. The fecal samples were then dried at 70°C for 72 h and finely ground to be able to pass through a 1-mm screen. All of the feed and feces samples were then frozen at -20°C until further analysis. The procedures utilized for the determination of DM, N and energy digestibility were conducted in accordance with the methods established by the AOAC (1998). Chromium levels were determined via UV absorption spectrophotometry (Shimadzu, UV-1201, Japan) and the apparent digestibility of DM, N and energy were calculated using indirect methods. The gross energy in the feed and feces was determined using a calorimeter (Mode 1241, Parr Instrument Co., USA).

For the blood characteristics assay, on the initial day of the experiment, blood samples were collected at cervical vein into both K_3EDTA vacuum tubes and clot activator vacuum tubes (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ, USA) from 2 pigs (1 male and 1 female) in each pen and the same pigs were sampled on the final day of the experiment. The concentrations of red blood cell (RBC) and white blood cell (WBC) counts in the whole blood were measured using the automatic blood analyzer (ADVIA 120, Bayer, USA). Besides, after collection, serum samples were subsequently centrifuged (2,000×g) for 30 minutes at 4°C. The blood urea nitrogen (BUN) was analyzed using the Abbott Spectrumurea nitrogen test (Series II, Abbot Laboratories, Dallas, TX), and the serum creatinine concentrations were determined using an Astra-8 Analyzer (Beckman Instruments, Inc., Brea, CA 92621). Plasma glucose concentrations were determined by colorimetric assay (Glucose Procedure #16-UV, Sigma Diagnostics, St. Louis, MO).

Exp. 2

Surgical procedures and animal care : The four barrow pigs had 7 d to adapt to their surroundings before the surgery. Pigs had *ad libitum* access to the same basal diet during this period. Feed was withheld from each animal 12 h before surgery, but the pigs had continuous access to water. In addition, anesthesia was induced by injecting the pigs with Strenial (Janssen Pharmaceutica, Belgium) and Zoletil 50 (Virbac Lab, Korea) before surgery. Pigs were surgically fitted with a simple T-cannula at approximately 10 cm cranial to the ileo-cecal junction, according to procedures adapted from Sauer et al. (1983). The cannulation site was cleaned daily with soft detergent and warm water during the entire experiment. The pigs were fed 2 meals daily until full feeding was achieved 4 d after surgery. A period of 10 d was allowed for surgical recovery before the beginning of the experiment.

Experimental design, diets and feeding : The four ileal-cannulated, growing barrows (20.17±1.31 kg) were housed in individual metabolism crates and randomly assigned to 1 of 4 treatments (same as Exp. 1) within a 4×4 Latin square design. The 4 feeding periods consisted of 4 d diet acclimation followed by 3-d ileal-digesta collection. The samples then were stored at -20°C until analysis. Ileal digesta were collected for 12 h periods between the morning and evening feeding (8:00 a.m. and 8:00 p.m.) on d 5, 6 and 7. After feeding, every 20 min the digesta were emptied into plastic containers and placed on ice. The daily feed allowance was $0.095\times BW^{0.75}$, as proposed by NRC (1998). Chromic oxide (0.20%) was added as an inert indicator to allow digestibility determination. The samples of digesta were then freeze-dried (-50°C) and finely ground for further analysis. Amino acids were analyzed by an amino acid analyzer (Biochrom 20, Pharmacia Biotech, England). Sulfur-containing amino acids were analyzed after cold performic acid oxidation overnight and subsequent hydrolysis.

Statistical analyses

In Exp. 1, all data were subjected to the GLM procedures of SAS (SAS Inst., 1996) as a randomized complete block design. Each pen served as the experimental unit. The initial BW was used as a covariate for ADFI and ADG, and initial value was used as a covariate for blood profile. In Exp. 2, the data were analyzed as 4×4 Latin square design using the GLM procedures of SAS (SAS Inst., 1996). Duncan's multiple range test was used to compare the means of the treatments (Ducan, 1955). Variability in the data was expressed as the pooled standard error (SE) and a $p<0.05$ was considered to be statistically significant.

RESULTS

Growth performance

During the overall period, ADG and G:F in pigs fed the NSPase1 diet were increased by 4.40% and 6.26% ($p<0.05$), respectively, compared with those fed the CON diet. There were no significant differences in ADG and G: F among the multienzyme treatments ($p>0.05$). Endopower did not affect performance and NSPase2 had no difference in performance compared with CON. No effects of dietary treatments on ADFI were observed ($p>0.05$) (Table 2).

Apparent nutrient digestibility

DM digestibility was greater ($p<0.05$) in ENDO

Table 2. Effects of multienzyme on growth performance in growing pigs[1]

Items	CON	ENDO	NSPase1	NSPase2	SE[2]
ADG (kg)	0.682[b]	0.696[ab]	0.712[a]	0.689[ab]	0.008
ADFI (kg)	1.255	1.239	1.235	1.241	0.013
Gain/feed	0.543[b]	0.562[ab]	0.577[a]	0.555[ab]	0.009

[1] CON = Control diet; ENDO = Control diet with 0.1% endopower; NSPase1 = Control diet with 0.1% NSPase; NSPase2 = Control diet with 0.2% NSPase.
[2] Pooled Standard error. [a, b] Means in the same row with different superscripts differ ($p<0.05$).

treatment group than that in CON group. Compared with CON group, N digestibility was increased ($p<0.05$) in multienzyme treatment groups during the experimental period. There were no statistical differences ($p>0.05$) in DM and N digestibility among multienzyme treatment groups. The multienzyme supplementation did not affect ($p>0.05$) the energy digestibility among treatments (Table 3).

Blood profiles

WBC, RBC and creatinine were not influenced ($p>0.05$) by the multienzyme supplementation (Table 4). However, BUN was higher ($p<0.05$) in ENDO treatment group compared with that in CON and NSPase1 treatment groups at the end of the experiment. The level of glucose on the final day of the experiment improved ($p<0.05$) in response to ENDO and NSPase2 treatments.

Apparent ileal amino acid digestibility

In Exp. 2, throughout the trial, supplementation of both Endopower and NSPase significantly improved ($p<0.05$) the apparent ileal digestibility (AID) of total EAA and NEAA compared with the CON group (Table 5). Besides, AID of total EAA and NEAA was higher ($p<0.05$) in NSPase1 and NSPase2 treatments than that in ENDO treatment. Overall, similar trend was observed in individual AID of AA with the exception of His, Lys and Val in EAA

Table 3. Effects of multienzyeme on nutrient digestibility in growing pigs[1]

Items (%)	CON	ENDO	NSPase1	NSPase2	SE[2]
DM	79.10[b]	82.01[a]	80.28[ab]	80.85[ab]	0.57
N	77.23[b]	80.83[a]	79.46[a]	79.62[a]	0.70
Energy	78.89	78.29	79.83	79.32	1.22

[1] CON = Control diet; ENDO = Control diet with 0.1% endopower; NSPase1 = Control diet with 0.1% NSPase; NSPase2 = Control diet with 0.2% NSPase.
[2] Pooled Standard error. [a, b] Means in the same row with different superscripts differ ($p<0.05$).

Table 4. Effects of multienzyme on blood profiles in growing pigs[1]

Items	CON	ENDO	NSPase1	NSPase2	SE[2]
WBC ($\times 10^3/\mu l$)					
Initial	19.81	18.89	19.98	20.07	1.230
Final	20.32	20.12	20.83	21.19	1.308
RBC ($\times 10^6/\mu l$)					
Initial	7.49	7.59	7.43	7.64	0.175
Final	7.55	7.68	7.61	7.56	0.104
Creatinine (mg/dl)					
Initial	1.34	1.38	1.46	1.34	0.048
Final	1.84	1.90	2.00	1.98	0.083
BUN (mg/dl)					
Initial	12.40	12.26	12.00	12.62	1.394
Final	17.54[b]	20.38[a]	17.68[b]	19.24[ab]	0.608
Glucose (mg/dl)					
Initial	96.80	94.40	93.80	95.20	3.706
Final	102.6[b]	112.2[a]	108.4[ab]	115.8[a]	2.666

[1] CON = Control diet; ENDO = Control diet with 0.1% endopower; NSPase1 = Control diet with 0.1% NSPase; NSPase2 = Control diet with 0.2% NSPase.
[2] Pooled Standard error. [a, b] Means in the same row with different superscripts differ ($p<0.05$).

Table 5. Effects of multienzyme on apparent ileal digestibility of amino acids in cannula pigs[1]

Items (%)	CON	ENDO	NSPase1	NSPase2	SE[2]
Essential amino acid					
ARG	86.30^{c}	92.70^{b}	93.12^{b}	97.10^{a}	0.66
HIS	56.55^{b}	66.01^{a}	71.18^{a}	68.14^{a}	2.38
ILE	80.75^{c}	86.56^{b}	90.33^{a}	87.92^{b}	0.70
LEU	81.82^{c}	85.97^{b}	90.74^{a}	90.43^{a}	1.89
LYS	81.35^{b}	87.55^{a}	88.92^{a}	88.80^{a}	0.91
MET	78.97^{b}	79.89^{b}	87.72^{a}	86.48^{a}	1.24
PHE	85.77^{b}	87.48^{b}	90.93^{a}	91.13^{a}	0.54
THR	72.07^{c}	79.67^{b}	84.25^{a}	80.59^{ab}	1.29
VAL	78.07^{c}	84.92^{ab}	87.61^{a}	83.21^{b}	1.10
Total EAA	77.96^{c}	83.41^{b}	87.20^{a}	85.98^{ab}	1.02
Non-essential amino acid					
ALA	71.26^{b}	79.04^{a}	84.22^{a}	80.25^{a}	1.58
ASP	76.13^{c}	85.71^{b}	87.92^{ab}	89.36^{a}	1.08
CYS	66.70^{b}	73.76^{ab}	80.03^{a}	81.10^{a}	3.43
GLU	84.58^{c}	89.97^{b}	91.69^{b}	95.87^{a}	0.69
GLY	67.14^{b}	78.71^{a}	80.74^{a}	79.96^{a}	1.45
PRO	68.97^{c}	73.48^{b}	88.41^{a}	89.26^{a}	1.19
SER	72.90^{c}	81.05^{b}	85.66^{a}	85.41^{a}	0.91
TYR	86.12^{bc}	83.77^{c}	89.44^{a}	86.88^{ab}	0.77
Total NEAA	74.22^{c}	80.68^{b}	86.01^{a}	86.01^{a}	1.26

[1] CON = Control diet; ENDO = Control diet with 0.1% endopower; NSPase1 = Control diet with 0.1% NSPase; NSPase2 = Control diet with 0.2% NSPase.

[2] Pooled Standard error. ^{a,b,c} Means in the same row with different superscripts differ ($p<0.05$).

and Ala, Cys and Gly in NEAA.

DISCUSSION

The results of the current study indicated that NSPase1 supplementation improved ADG and G:F but had no influence on ADFI, with no significant differences observed among multienzyme treatment groups. Our findings are consistent with a study conducted by Omogbenigun et al. (2004), who reported that an improvement in ADG and G:F had been observed in piglets fed diets based on corn and wheat supplemented with a enzyme cocktail containing cellulase, galactanase, mannanase, and pectinase. Significant benefits to xylanase supplementation were demonstrated in growing pigs fed corn-based diets in terms of growth rate (Schulze and Campbell, 1998; Fang, 2007). Besides, Kim et al. (2001a) observed that ADG and G:F was increased by 3 and 7%, respectively, with 0.1% enzyme complex (the same Endopower used in our experiment) supplementation to corn and SBM based diets for weaned piglets. In continued study, Kim et al. (2001b) found the enzyme supplementation at 0.05% level (Endopower) in a diet with 5% low ME showed similar performance with those pigs in positive control diet. However, the results are not always consistent. Some studies failed to observe a positive effect of carbohydrases supplementation on growth performance (Grandhi, 2001; Olukosi, 2007). The contradictions in the impact of multienzyme supplementation on growth performance may be attributed to the differences in the diets composition, the age of pigs used. In addition, the enzyme source, the situations under which the specific ingredient was grown, the storage and process of feed, the interactions among dietary compositions and health status may also exert a significant effect on growth performance (Kim, 2003a, b). Besides, high dose rate of NSPase did not improve performance and this may indicate the enzyme was dose dependent. We hypnotized that the lack of benefits in high dosage treatments might be due to the amount of substrates which were not enough for more enzyme. But the exact reason was not clear.

Multienzyme supplementation increased DM and N digestibility while failing to improve energy digestibility in the present study. Some authors demonstrated the enzyme additions could exert a positive effect on nutrient digestibility (Omogbenigun et al., 2004; Fang, 2007). Yin et al. (2001) reported that xylanase supplementation significantly increased both ileal and overall digestibility of nutrients. Conversely, other studies failed to observe an improvement in nutrient digestibility (Petty et al., 2002;

Olukosi, 2007). In addition, the increase in N digestibility in our experiment may partially mirror the improvement in the growth performance. The two multienzymes used in this study were aimed at degrading NSP known as anti-nutritive factors (mainly insoluble arabinoxylans in corn and α-galactosides and β-mannans in SBM dietary components, respectively) in corn and SBM based diets. In the herein study, the improved growth performance and N digestibility following NSPase1 supplementation may be primarily due to a result of successful degradation of NSP. This is in agreement with previous researches (Meng et al., 2002). We found that most of AID of AA was increased by the multienzyme additions. In previous researches, the effect of exogenous enzymes supplementation on AID of AA appeared to be contradictory in pigs. Our findings agreed well with Kim et al. (2001b, 2006) who revealed that most AID of AA was increased by the enzyme complex (Endopower). Besides, Yin et al. (2001) observed β-glucanase improved ileal digestibility of CP and most AA. In contrast, Ji et al. (2008) indicated that β-glucanase-protease enzymes blend improved the fecal proximate digestibility but standardized ileal AA digestibility was not increased by the enzyme blend in corn-SBM-based growing pig diets. Previous studies indicated that fiber level and type can affect AID of AA by increasing endogenous N and AA losses while fiber degrading enzymes have been used successfully to improve AID of AA and/or to decrease endogenous AA losses (Lewis, 2001). Yin et al. (2001) explained that NSP backbones can be hydrolyzed by exogenous enzymes and this can ease enzyme access to the AA originally encapsulated by the carbohydrate complex. Meanwhile, the excretion of endogenous nitrogen was decreased by the hydrolysis of dietary fiber. NSPase is more effective in AA digestibility than Endopower, but growth performance did not reflect such difference in this study. This may indicate that the increase in AA digestibility cannot contribute to the improvement of growth performance. Overall, the current results showed that even in the corn and SBM diets which owned low-fiber content and high nutrient digestibility, exogenous enzymes supplementation still have the potential to improve the digestibility of DM, N and AA.

Urea excreted in urine is the main nitrogenous end-product produced by AA catabolism in pigs, and its concentration is directly related to the rate of urea synthesis, protein type, intake and quality (Bassily et al., 1982). In the present study, BUN in ENDO treatment group was significantly higher than that in other treatments. This indicates that the addition of ENDO may result in a diet with the different protein digestibility from the control diet (corn-SBM). The improvement in N and AA digestibility in the current trial may partially reflect this difference. To our best knowledge, few experiments have been conducted to compare the BUN coefficients of corn-SBM diets. Wang et al. (2009) reported that BUN was not affected by the addition of the enzyme cocktail (α-1,6-β-galactosidse, β-1,4-mannanase, and β-1,4-mannosidase) to low-nutrient corn-SBM containing DDGS diets. Therefore, more studies are needed to investigate the amino acid digestibility and amino acid composition in carcass of pigs fed the experimental diets used in this study. We observed an increase in the glucose concentration among enzyme supplementation treatments.

In conclusion, NSPase1 supplementation was effective in enhancing ADG and G:F. N digestibility was improved by the enzyme additions. Besides, the levels of glucose were also affected by multienzyme supplementation.

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