



# Effects of dietary carbohydrases on productive performance and immune responses of lactating sows and their piglets

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## Abstract

This study was conducted to evaluate effects of dietary multi-carbohydrases (MCS) in a lactating sow diet on productive performance and immune responses of sows and their piglets. A total of 12 sows (218.37 ± 5.5 kg BW; 2 parity) were randomly assigned to 2 dietary treatments: a diet based on corn-soybean meal (CON) and CON with 0.01% MCS. The MCS contained xylanase (2,700 units/g), β-glucanase (700 units/g), and cellulase (800 units/g). Sows were fed the dietary treatments for 28 days (weaning) after farrowing. Blood samples were collected from sows on d 0, 3, and 7 after farrowing and randomly selected 2 nursing piglets in each sow on d 3, 7, and 14 after birth. Measurements were productive performance of sows, frequency of diarrhea of piglets, and immune responses of sows and their piglets. Sows fed MCS had lower ( $p < 0.05$ ) their body weight change than those fed CON. Piglets from sows fed MCS had higher ( $p < 0.05$ ) average weight gain and body weight at weaning day and lower ( $p < 0.10$ ) frequency of diarrhea than those from sows fed CON. Sows fed MCS had lower number of white blood cells (WBC) on d 3 ( $p < 0.05$ ) and TGF-β1 on d 7 ( $p < 0.10$ ) during lactation than those fed CON. Similarly, piglets from sows fed MCS had also lower ( $p < 0.05$ ) number of WBC on d 3 and d 7 and TGF-β1 and C-reactive protein on d 7 during lactation than those from sows fed CON. In addition, piglets from sows fed MCS had higher ( $p < 0.10$ ) immunoglobulin G and M on d 7 during lactation those from sows fed CON. In conclusion, addition of dietary MCS in the lactating sow diet based on corn and soybean meal improved productive performance of sows and their litters and modulated their immune responses.

**Keywords:** Carbohydrase, Immune responses, Lactating sows and their litter, Productive performance

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## Background

Corn and soybean meal (SBM) are main ingredients as energy and protein sources, respectively, in swine diets. In addition, they contain non-starch polysaccharides (NSPs) such as arabinoxylans, xylans, phytate, glucans, and cellulose that directly affect the negative impact on nutrient and energy digestibility [1,2]. Generally, yellow dent corn and soybean meal used in swine diets have 9.7% and 21.7% of NSPs [3]. These NSPs cannot be digested in the small intestine of pigs because pigs do not produce digestive enzymes to degrade NSPs and reach the large intestine to be sources of fermentable materials for microbiota of pigs [4–6]. Therefore, swine industry has been looking for the ways to improve and optimize the availability and digestibility of the NSPs.

Addition of carbohydrases in swine diets may be one of solutions to improve the availability of NSPs. Dietary carbohydrase, such as xylanase, glucanase, or cellulase is an enzyme that breaks down carbohydrates into sugars and is usually produced by bacterial and fungal species such as bacilli, asperigilli, or etc. [7,8]. Dietary carbohydrase can improve the digestion and absorption of nutrients by changing digesta viscosity and by reducing anti-nutritional factors from NSPs in animal diets [9–13].

Previous studies showed addition of dietary carbohydrases in grower diets [13,14] and weaner diet [15] increased growth performance and nutrient digestibility. In addition, dietary cellulase in sow diet improved nutrient digestibility of sows and growth rate of their litters [16]. However, there are limited information about dietary carbohydrases on productive performance of sows in lactation diets. Therefore, the objective of this study was to evaluate effects of carbohydrases in sow diets during lactation on productive performance and immune responses of lactating sows and their litters.

## Materials and Methods

The experimental protocol for this study was reviewed and approved by the Animal Care and Use Committee of Chungnam National University, Daejeon, Korea. This study was conducted at the facility of animal research center of Chungnam National University.

### Experimental design, animals, and diets

Twelve lactating sows (Landrace × Yorkshire; 218.37 ± 5.5 kg of average body weight [BW]; 2.0 of average parity) were used in this study. On d 110 of gestation, sows were moved to farrowing crates with an individual feeder and drinker and randomly allotted to 2 diets in a completely randomized design: 1) a general diet based on corn and soybean meal (CON) and 2) CON supplemented with 0.01% of multi-carbohydrases (MCS). The MCS (DSM Nu-

trition Korea Ltd., Seoul, Korea) contained xylanase (2,700 units/g), β-glucanase (700 units/g), and cellulase (800 units/g). The diets were formulated to meet or exceed the NRC (2012) estimates of nutrient requirements of lactating sows (Table 1). Sows were fed 3.0 kg of the diets from d 110 of gestation to farrowing and had *ad-libitum* access of the diets from farrowing to weaning. Water was freely accessible during entire experimental period.

### Measurements and sample collection

The weaning days of piglets was at 28.7 ± 1.8 d of age and the number of born alive and died piglets were also recorded. The feed intake per sow was recorded during lactation and average daily feed intake was calculated. The BW of sow and nursery piglets were measured at farrowing and weaning and average BW change of sow and average BW gain of piglets were calculated. Backfat depth of sow was measured at the P2 position (65 mm down the left side from the midline at the same level as the last rib curve) us-

**Table 1. Composition of experimental diets during lactation (as-fed basis)**

| Basal diet                      |       |
|---------------------------------|-------|
| Ingredient (%)                  |       |
| Corn                            | 65.54 |
| Soybean meal (45%)              | 31.81 |
| Limestone                       | 0.85  |
| Mono-dicalcium phosphate        | 1.40  |
| Vitamin premix <sup>1</sup>     | 0.20  |
| Mineral premix <sup>2</sup>     | 0.20  |
| Total                           | 100   |
| Calculated chemical composition |       |
| Metabolizable energy (Mcal/kg)  | 3.43  |
| Crude protein (%)               | 19.76 |
| Crude fat (%)                   | 2.86  |
| Crude fiber (%)                 | 3.33  |
| Neutral detergent fiber (%)     | 10.78 |
| Acid detergent fiber (%)        | 4.63  |
| Calcium (%)                     | 0.75  |
| Phosphorus (%)                  | 0.65  |
| Lysine (%)                      | 1.02  |
| Methionine (%)                  | 0.30  |
| Threonine (%)                   | 0.74  |
| Tryptophan (%)                  | 0.22  |

<sup>1</sup>The vitamin premix provided the following quantities of vitamins per kilogram of diet: vitamin A, 10,000 IU; vitamin D<sub>3</sub>, 2,000 IU; vitamin E, 48 IU; vitamin K<sub>3</sub>, 1.5 mg; riboflavin, 6 mg; niacin, 40 mg; D-pantothenic acid, 17 mg; biotin, 0.2 mg; folic acid, 2 mg; choline, 166 mg; vitamin B<sub>6</sub>, 2 mg; and vitamin B<sub>12</sub>, 28 µg.

<sup>2</sup>The mineral premix provided the following quantities of mineral per kilogram of diet: Fe, 90 mg from iron sulfate; Cu, 15 mg from copper sulfate; Zn, 50 mg from zinc oxide; Mn, 54 mg from manganese oxide; I, 0.99 mg from potassium iodide; Se, 0.25 mg from sodium selenite.

ing a real-time ultrasound scanner (Anyscan BF, SongKang GLC Co., Seongnam, Korea) at farrowing and weaning. The presence of diarrhea from each piglet was checked visually each day for lactation and recorded with two scales: the presence of diarrhea or not. Frequency of diarrhea was calculated by counting piglet days with the presence of diarrhea.

Blood samples were collected from a jugular vein of each sow on d 0, 3, and 7 of lactation and randomly selected two piglets (1 barrow and 1 gilt) per sow on d 3, 7, and 14 of lactation using EDTA tubes (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ, USA) with anticoagulant and serum tubes.

### Measurements of white blood cells and serum cytokines, acute phase protein, cortisol, and immunoglobulin G, M, and A

The number of white blood cells (WBC) was analyzed using an automated hematology analyzer calibrated for porcine blood (scil Vet abc hematology analyzer, scil animal care company, F-67120 Altorf, France). The serum tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ; R&D Systems, Inc., Minneapolis, MN, USA), transforming growth factor- $\beta$  1 (TGF- $\beta$  1; R&D Systems, Inc., Minneapolis, MN, USA), C-reactive protein (CRP; Abnova Corp., Taipei City,

Taiwan), cortisol (Cusabio, Wuhan, China), and immunoglobulin G, M, and A (IgG, IgM, and IgA; Abnova Corp., Taipei City, Taiwan) were measured using porcine ELISA kits and following the procedure of manufacturer.

### Statistical analyses

Data were analyzed using the PROC GLM procedure of SAS (SAS Inst. Inc., Cary, NC, USA) in a completely randomized design. The experimental unit were sow and litter. The statistical model for productive performance and immune responses of sow and litter included effects of dietary treatment and parity as a covariate. The  $\chi^2$  test was used for the frequency of diarrhea. Results were presented as means and standard error of the mean. Statistical significance and tendency were considered at  $p < 0.05$  and  $0.05 \leq p < 0.10$ , respectively.

## Results and Discussion

Sows fed MCS had less ( $p < 0.05$ ) body weight loss during lactation than those fed CON, but there were no differences on other productive performances of lactating sows between CON and MCS (Table 2). Regarding longevity of sows, sows need to

**Table 2.** Productive performance of lactating sows fed dietary treatments

| Item   | Treatments |        | SEM    | p-value |
|--|------------|--------|--------|---------|
|  | CON        | MCS    |        |         |
| Sows (n)   | 6          | 6      | -      | -       |
| Parity   | 2.1        | 2.1    | 0.041  | 0.283   |
| Weaning days (d)                                   | 29.00      | 28.33  | 0.767  | 0.553   |
| Feed intake (kg)                                   | 183.28     | 182.45 | 2.345  | 0.785   |
| ADFI (kg)  | 6.32       | 6.44   | 0.119  | 0.491   |
| Initial BW (kg)                                    | 254.92     | 248.13 | 14.964 | 0.755   |
| Final BW (kg)                                      | 239.45     | 240.28 | 14.583 | 0.969   |
| BW change (kg)                                     | -15.47     | -7.85  | 1.525  | < 0.05  |
| Initial backfat depth (mm)                         | 20.83      | 21.26  | 0.379  | 0.446   |
| Final backfat depth (mm)                           | 18.10      | 18.66  | 0.395  | 0.340   |
| Backfat depth change (mm)                          | 2.73       | 2.60   | 0.295  | 0.753   |
| Born alive piglets (n)                             | 10.53      | 10.81  | 1.051  | 0.850   |
| Died piglets (n)                                   | 0.67       | 0.87   | 0.380  | 0.592   |
| Weaned piglets (n)                                 | 9.83       | 9.96   | 1.046  | 0.761   |
| BW of born piglets (kg)                            | 1.61       | 1.61   | 0.08   | 0.970   |
| BW of weaned piglets (kg)                          | 6.93       | 7.55   | 0.175  | < 0.05  |
| Average BW gain of piglets (g/d)                   | 183.49     | 210.91 | 8.100  | < 0.05  |
| Frequency of diarrhea of piglets (%) <sup>1)</sup> | 9.75       | 8.63   | -      | < 0.10  |

Values are presented as the least squares mean of 6 replicates.

<sup>1)</sup>Frequency of diarrhea of nursery pigs = (number of diarrhea/number of nursery pigs)  $\times$  100. Data was analyzed by the  $\chi^2$  test.

CON, control diet based on corn and soybean meal; MCS, multi-carbohydrate diet; SEM, standard error of means; ADFI, average daily feed intake; BW, body weight.

recover their feed intake during lactation as soon as possible after farrowing because lactating sows use more their energy to produce milk than to recover and/or maintain their body condition for next reproduction [17,18]. Due to the energy use of lactating sows, they generally lose their body weight and backfat depth [19,20]. However, the present study showed dietary MCS contributed to less body weight loss of lactating sows because dietary MCS may break down NSPs pigs cannot generally use into available sugars, resulting in improved utilization of nutrients to make more energy. In addition, previous study showed addition of NSP degrading enzymes in pig diets containing high NSP reduced negative effects of NSP and affected nutrient digestibility and performance of pigs positively [21,22].

Sows fed MCS had lower number of WBC on d 3 ( $p < 0.05$ ) and TGF- $\beta$  1 on d 7 ( $p < 0.10$ ) during lactation than those fed CON, but MCS did not modify concentration of TNF- $\alpha$ , CRP, and cortisol of lactating sows compared with CON (Table 3). Sow farrowing is the stressful event that can negatively affect productive performance and increase inflammation by modulating immune system of lactating sows after farrowing [23,24]. It is important for lactating sows to recover the stress soon after farrowing for maintenance of their body condition and nursing their litter normally. The present study showed dietary MCS decreased WBC and TGF-

$\beta$  1 within 7 days after farrowing. It may indicate dietary MCS contributes to attenuation of inflammation caused by the stress from farrowing, resulting in fast recovery of lactating sows from the stress. Previous study reported that dietary carbohydrases could change substrates for gut microbes, especially beneficial microbes, by degrading NSPs in pig diets and it could affect gut health and immunity of pigs positively and indirectly [25]. Moreover, another studies indicated that the substrates from NSPs degradation by dietary carbohydrases, such as short chain oligosaccharides, could modulate immunity of pigs like prebiotic effects directly [26–28].

Piglets from sows fed MCS had higher ( $p < 0.05$ ) average weight gain and body weight at weaning day (Table 2), less ( $p < 0.10$ ) frequency of diarrhea (Table 2), lower ( $p < 0.05$ ) number of WBC on d 3 and d 7 and TGF- $\beta$  1 and CRP on d 7 during lactation (Table 4), and higher ( $p < 0.10$ ) IgG and IgM on d 7 during lactation (Table 5) than those from sows fed CON. However, no differences were found on concentration of TNF- $\alpha$  and cortisol between CON and MCS (Table 4). The beneficial effects of dietary MCS on performance and immune responses of lactating sows mentioned above may save more energy for sows to make more milk and may make sows faster recovery and healthy. These potential benefits may make nursery piglets bigger and healthy by improving average body weight gain, reducing frequency of diar-

**Table 3. Effects of multi-carbohydrases on number of WBC, serum TNF- $\alpha$ , TGF- $\beta$ 1, CRP and cortisol of lactating sows**

| Item                              | Treatments |        | SEM    | p-value |
|-----------------------------------|------------|--------|--------|---------|
|                                   | CON        | MCS    |        |         |
| Day 0                             |            |        |        |         |
| WBC ( $\times 10^3/\mu\text{L}$ ) | 15.02      | 14.48  | 1.016  | 0.718   |
| TNF- $\alpha$ (pg/mL)             | 265.63     | 313.21 | 19.827 | 0.121   |
| TGF- $\beta$ 1 (pg/mL)            | 719.73     | 742.90 | 74.003 | 0.829   |
| CRP (ng/mL)                       | 260.77     | 195.50 | 31.604 | 0.175   |
| Cortisol (ng/mL)                  | 2.51       | 2.58   | 0.206  | 0.821   |
| Day 3                             |            |        |        |         |
| WBC ( $\times 10^3/\mu\text{L}$ ) | 13.70      | 11.37  | 0.614  | < 0.05  |
| TNF- $\alpha$ (pg/mL)             | 267.52     | 257.77 | 6.617  | 0.322   |
| TGF- $\beta$ 1 (pg/mL)            | 785.20     | 704.56 | 69.273 | 0.430   |
| CRP (ng/mL)                       | 193.08     | 186.32 | 31.313 | 0.882   |
| Cortisol (ng/mL)                  | 2.88       | 2.52   | 0.221  | 0.301   |
| Day 7                             |            |        |        |         |
| WBC ( $\times 10^3/\mu\text{L}$ ) | 12.60      | 12.28  | 0.695  | 0.754   |
| TNF- $\alpha$ (pg/mL)             | 279.33     | 252.36 | 26.023 | 0.740   |
| TGF- $\beta$ 1 (pg/mL)            | 951.14     | 730.61 | 83.832 | < 0.10  |
| CRP (ng/mL)                       | 167.75     | 127.49 | 35.199 | 0.438   |
| Cortisol (ng/mL)                  | 2.59       | 2.43   | 0.333  | 0.743   |

Values are presents as the least squares mean of 6 replicates for lactating sows.

WBC, white blood cells; TNF- $\alpha$ , tumor necrosis factor- $\alpha$ ; TGF- $\beta$ 1, transforming growth factor- $\beta$ 1; CRP, C-reactive protein; CON, control diet based on corn and soybean meal; MCS, multi-carbohydrase diet; SEM, standard error of means.

**Table 4.** Effects of multi-carbohydrases on number of WBC, serum TNF- $\alpha$ , TGF- $\beta$ 1, CRP and cortisol of nursing piglets

| Item                              | Treatments |          | SEM     | p-value |
|-----------------------------------|------------|----------|---------|---------|
|                                   | CON        | MCS      |         |         |
| Day 3                             |            |          |         |         |
| WBC ( $\times 10^3/\mu\text{L}$ ) | 10.69      | 9.17     | 0.337   | < 0.01  |
| TNF- $\alpha$ (pg/mL)             | 264.14     | 282.11   | 7.132   | 0.571   |
| TGF- $\beta$ 1 (pg/mL)            | 742.15     | 723.24   | 79.028  | 0.869   |
| CRP (ng/mL)                       | 50.81      | 64.95    | 6.820   | 0.173   |
| Cortisol (ng/mL)                  | 2.10       | 2.10     | 0.066   | 0.999   |
| Day 7                             |            |          |         |         |
| WBC ( $\times 10^3/\mu\text{L}$ ) | 17.77      | 13.22    | 1.109   | < 0.01  |
| TNF- $\alpha$ (pg/mL)             | 290.39     | 290.43   | 18.321  | 0.999   |
| TGF- $\beta$ 1 (pg/mL)            | 869.89     | 757.52   | 33.774  | < 0.05  |
| CRP (ng/mL)                       | 128.65     | 92.63    | 9.349   | < 0.05  |
| Cortisol (ng/mL)                  | 2.26       | 2.24     | 0.139   | 0.911   |
| Day 28 (weaning day)              |            |          |         |         |
| WBC ( $\times 10^3/\mu\text{L}$ ) | 16.74      | 15.74    | 1.507   | 0.649   |
| TNF- $\alpha$ (pg/mL)             | 358.61     | 348.65   | 16.150  | 0.672   |
| TGF- $\beta$ 1 (pg/mL)            | 1,058.97   | 1,055.12 | 135.405 | 0.984   |
| CRP (ng/mL)                       | 110.16     | 78.47    | 20.732  | 0.305   |
| Cortisol (ng/mL)                  | 4.24       | 4.09     | 0.306   | 0.735   |

Values are presented as the least square mean of 6 replicates for nursing piglets.

WBC, white blood cells; TNF- $\alpha$ , tumor necrosis factor- $\alpha$ ; TGF- $\beta$ 1, transforming growth factor- $\beta$ 1; CRP, C-reactive protein; CON, control diet based on corn and soybean meal; MCS, multi-carbohydrase diet; SEM, standard error of means.

**Table 5.** Effects of multi-carbohydrases on serum immunoglobulin G, M, and A of nursing piglets

| Item        | Treatments |        | SEM    | p-value |
|-------------|------------|--------|--------|---------|
|             | CON        | MCS    |        |         |
| Day 3       |            |        |        |         |
| IgG (ng/mL) | 58.62      | 51.75  | 7.261  | 0.519   |
| IgM (ng/mL) | 18.51      | 21.89  | 4.887  | 0.636   |
| IgA (ng/mL) | 11.95      | 5.55   | 38.948 | 0.914   |
| Day 7       |            |        |        |         |
| IgG (ng/mL) | 247.04     | 278.37 | 10.493 | < 0.10  |
| IgM (ng/mL) | 22.22      | 29.00  | 2.383  | < 0.10  |
| IgA (ng/mL) | 81.81      | 71.55  | 26.409 | 0.789   |

Values are presented as the least square mean of 6 replicates for piglets.

CON, control diet based on corn and soybean meal; MCS, multi-carbohydrase diet; SEM, standard error of means; IgG, immunoglobulin G; IgM, immunoglobulin M; IgA, immunoglobulin A.

rhea, and attenuating immune responses.

## Conclusion

In conclusion, addition of dietary MCS in the lactating sow diet based on corn and soybean meal improved productive performance and attenuated immune responses of lactating sows and their litters.

## Competing interests

No potential conflict of interest relevant to this article was reported.

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**Availability of data and material**

Upon reasonable request, the datasets of this study can be available from the corresponding author.

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**Ethics approval and consent to participate**

The experimental protocol for this study was reviewed and approved by the Animal Care and Use Committee of Chungnam National University (Approval# CNU-00611).

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