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Original research article

Dose-response effects of in-feed antibiotics on growth performance and nutrient utilization in weaned pigs fed diets supplemented with yeast-based nucleotides

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ABSTRACT

Dietary nucleotides are bioactive compounds with the potential to mitigate weaning-associated challenges in piglets. An experiment was conducted to determine the interaction effect of antimicrobial growth promoters (AGP) and a nucleotide-rich yeast extract (NRYE) on growth performance and apparent total tract digestibility (ATTD) of dry matter (DM), crude protein (CP) and gross energy (GE), and to establish whether NRYE supplementation may completely or partially replace AGP in diets for weaned pigs. In phase 1 and 2, corn, wheat, canola meal and soybean meal based diets, which were formulated to contain 0.0 or 0.1% NRYE with 0, 25, 50, 75 or 100% of the recommended AGP dosage, were fed to 108 twenty-one day old piglets (initial body weight 7.11 ± 0.9 kg; mean ± SD) from d 1 to 14 and 15 to 28, respectively. Overall, increasing AGP level in NRYE supplemented diets linearly decreased average daily gain (ADG) (P = 0.002) and gain-to-feed ratio (G:F) (P = 0.007); and quadratically decreased ATTD of DM (P = 0.001), CP (P = 0.003) and G:F (P = 0.017) during phase 2. Compared with control and pigs fed NRYE with 100% of recommended AGP dosage, pigs fed 0.1% NRYE without AGP had greater (P < 0.05) ADG and G:F in phase 2 and overall. In conclusion, supplementing 0.1% NRYE improved growth performance of pigs but this beneficial effect was reduced by increasing dietary AGP dosage.

1. Introduction

Sub-therapeutic levels of antimicrobial growth promoters (AGP) have been supplemented in pig starter diets to mitigate proliferation of enteropathogenic bacteria associated with post-weaning diarrhea (Heo et al., 2013). However, public pressure to eliminate the use of in-feed AGP from livestock feed has intensified because of their potential association with the development of microbial antibiotic resistance in humans and livestock (Heuer et al., 2006), and environmental contamination (Carlson et al., 2004). Furthermore, pork products sourced from pigs raised under AGP-free regimen are on high demand in several major international markets. Therefore, there is an urgent need for viable alternatives to dietary antibiotics (Choct, 2001; Heo et al., 2013; Gong et al., 2014).

Dietary nucleotides have been suggested as a potential alternative therapy to in-feed AGP in mitigating post-weaning stress in piglets. Sauer et al. (2011) reviewed the role of dietary nucleotides in piglets and showed that they may have beneficial effects on intestinal morphology and function, intestinal microbiota, immune function, nutrient metabolism, and hepatic morphology and function. Zomborszky-Kovacs et al. (2000) and Weaver and Kim (2014) reported that dietary nucleotides supplementation improved the growth performance of weaned piglets. However, studies that simultaneously compare the effectiveness of dietary nucleotides and AGP are lacking thereby making it difficult to substantiate the role of nucleotides as alternatives to AGP.

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Nucleotides can be supplemented in pure forms or as part of a nucleotide-rich yeast extract (NRYE), the latter being more affordable than the former.

The aim of this study was to determine the effect of supplementing 0.1% NRYE to diets containing graded levels of AGP on piglet growth performance and apparent total tract digestibility (ATTD) of dry matter (DM), crude protein (CP) and gross energy (GE), and to establish whether NRYE supplementation may completely or partially replace AGP in piglet starter diets.

2. Materials and methods

All experimental procedures were reviewed and approved by the University of Manitoba Animal Care Committee, and pigs were handled in accordance with the guidelines described by the Canadian Council on Animal Care (2009).

2.1. Animals and housing

One-hundred and eight (Duroc × [Yorkshire × Landrace]) male and female piglets, weaned at 21 d and with an initial average body weight of 7.11 ± 0.9 kg were used in a 4-wk study. The pigs were housed in an environmentally controlled nursery building with pens equipped with a feeder, a nipple drinker, and plastic-covered expanded metal floors. The room temperature was maintained at 29 ± 1°C in wk 1, and then gradually decreased by 1°C every week thereafter.

2.2. Experimental design

On d 1, pigs were randomly assigned to the dietary treatments based on the initial body weight with 3 pigs per pen and 6 replications per treatment. Piglets were fed a standard phase 1 and 2 diet (Table 1) without or with 0.1% NRYE from d 1 to 14, and d 15 to 28, respectively. Diets with 0.1% NRYE were supplemented with 0, 25, 50, 75 and 100% of the recommended AGP dosage in each phase. The NRYE supplement, Maxi-Gen Plus, was supplied by Canadian Bio-systems Inc. (Calgary, AB, Canada) and contained cell wall polysaccharides (21.6%), CP (32.7%), carbohydrates (14.3%) and a mixture of 5 nucleotides (1.1%; adenosine monophosphate, cytosine monophosphate, inosine monophosphate, uridine monophosphate and guanosine monophosphate), with 1 g of the NRYE additive supplying approximately 0.1% of mixed nucleotides. The AGP supplements, Aueromycin and Tiamulin, were supplied by Bio Agri Mix LP (Mitchell, ON, Canada) and were included following the Canadian Food Inspection Agency recommended dosage of 55 mg of Aueromycin (chlortetracycline) and 31.2 mg of Tiamulin per kg of diet. All diets were fed as mash and contained 0.3% titanium dioxide as an indigestible marker. Feed disappearance and body weight were recorded weekly. Representative freshly voided fecal samples were collected over the last 3 d of wk 2 and 4, and stored at −20 °C until required for analysis.

2.3. Sample preparation and chemical analyses

Fecal samples were dried in an oven at 60 °C for 4 d and pooled for each pen and week of collection and along with diet samples, they were finely ground to pass through a 1 mm screen using a Cyclotec 1093 Sample Mill (FOSS North America, Eden Prairie, MN, USA), and Thomas-Wiley mill (Thomas Scientific Swedesboro, NJ, USA), respectively, and thoroughly mixed before being analyzed for DM, GE, CP, and titanium dioxide.

Dry matter was determined according to AOAC (1990) method 925.09 and GE was determined using an adiabatic oxygen bomb calorimeter (Parr Instrument Co., Moline, IL, USA) which had been calibrated using benzoic acid as a standard. Crude protein (N × 6.25) was determined according to method 990.03 of AOAC (1990) using a combustion analyzer (model CNS-2000; Leco Corp., St. Joseph, MI, USA). Samples for TiO_2 were ashed and digested as described by (Lomer et al., 2000) and were measured by inductively coupled plasma mass spectrometer (Varian Inc., Palo Alto, CA, USA).

Table 1
Ingredient and chemical composition of the basal diets (as-fed basis).

Item	Phase 1[1]	Phase 2[2]
Ingredient, %		
Corn	25.90	38.35
Wheat	13.75	21.90
Canola meal	10.00	10.00
Soybean meal	20.00	23.85
Fish meal	5.00	0.00
Dried whey	20.00	0.00
Vegetable oil	2.50	2.35
Limestone	0.60	0.80
Monocalcium phosphate	0.60	1.00
Iodized salt	0.25	0.25
Vitamin-trace mineral premix[3]	1.00	1.00
L-Lys · HCl	0.10	0.20
Titanium dioxide	0.30	0.30
Calculated nutrient content		
DE, kcal/kg	3,472	3,450
CP, %	22.7	20.9
Ca, %	0.80	0.70
Total P, %	0.70	0.69
Available P, %	0.50	0.33
SID[4] AA, %		
Lys	1.40	1.24
Met	0.40	0.34
Thr	0.90	0.78
Analyzed composition, %		
CP	22.1	20.8
Ca	0.90	0.79
Total P	0.75	0.70

[1] Phase 1 diet was fed to pigs from d 1 to 14.
[2] Phase 2 diet was fed to pigs from d 15 to 28.
[3] Premix provided per kilogram of complete diet: 9,000 IU of vitamin A; 1,500 IU of vitamin D_3; 18 mg of vitamin E; 1.5 mg of vitamin K; 250 mg of choline; 30 mg of niacin; 27.5 mg of calcium pantothenate; 9.4 mg of riboflavin; 2 mg of pyridoxine; 25 μg of cyanocobalamin; 80 μg of biotin; 0.5 mg of folic acid; 18 mg of Cu from copper sulfate, 110 mg of Zn from zinc oxide, 0.2 mg of I from calcium iodide, 110 mg of Fe from ferrous sulfate, 50 mg of Mn from manganese dioxide, and 0.3 mg of Se from sodium selenite.
[4] SID = standardized ileal digestible.

2.4. Calculations and statistical analysis

The digestibility of nutrients were calculated using the following equation: apparent nutrient digestibility (%) = $\{1-[(T_d/T_f) \times (N_f/N_d)]\} \times 100$, where T_d and T_f are the titanium dioxide concentration in the diet and feces, respectively, and N_f and N_d are the nutrient concentration in the feces and diet, respectively. Data were analyzed using the mixed procedure of SAS (SAS Inst., Inc., Cary, NC). Initial body weight was used as a covariate for analyses of ADG data. Pre-planned contrasts were used to compare pigs receiving 0.1% NRYE with control and those offered 100% recommended AGP dosage. Linear and quadratic effects were determined using orthogonal polynomial contrasts and differences were considered significant at $P < 0.05$.

3. Results and discussion

The 0.1% inclusion level of NRYE was selected based on the study of Martinez-Puig et al. (2007) and preliminary studies in our laboratory (Waititu et al., 2013) showing that supplementing NRYE at levels above 0.1% does not have any added benefits to the performance of piglets.

In phase 2 and overall, pigs fed 0.1% NRYE without AGP had higher ($P < 0.05$) ADG and G:F than control pigs. This observation contradicts the studies of Lee et al. (2007) who supplemented 0.7% NRYE and Martinez-Puig et al. (2007) who supplemented 0.7 and 1.0% NRYE and reported no effect of supplementing NRYE on growth performance. However, the results support the studies of Zomborszky-Kovacs et al. (2000) who supplemented pure uracil and adenine at 0.5% each and Weaver and Kim (2014) who supplemented a mixture of nucleotides high in inosine at 0.02, 0.05, or 0.1% inclusion level and reported significant improvement in growth performance. Nucleotides are thought to improve performance responses in piglets by enhancing the development of the immune cells and the intestinal mucosa because they are building blocks of DNA, RNA and ATP, which are required during cell division (Grimble and Westwood 2001; Sauer et al., 2011). Dietary supplementation of nucleotides is more important for enterocytes and other cells with a high rate of replication and a low level of *de novo* nucleotide synthesis (Cosgrove, 1998). By enhancing cell growth, the intestinal mucosa and immune system mature faster thus reducing the stress that the animals may suffer as a result of various enteric diseases or post-weaning stress. Additionally, because nucleotides aid in tissue development, their effect is considered long-lasting (Singhal et al., 2010).

During phase 2 and overall, pigs fed 0.1% NRYE without AGP had greater ($P < 0.05$) ADG and G:F than pigs fed 0.1% NRYE with 100% of recommended AGP dosage (Table 2). This implies that increasing AGP level in NRYE supplemented diets suppressed the beneficial effects of NRYE. This is further supported by the results showing that increasing AGP level in diets with 0.1% NRYE linearly decreased ADG and G:F during phase 2 ($P = 0.002$ and 0.007, respectively) and overall ($P = 0.002$ and 0.006, respectively). To the best of our knowledge, we could not find studies to compare with these observations because studies that simultaneously examine the effect of AGP and NRYE are lacking.

The beneficial effect of AGP was observed in phase 1 showing that increasing AGP level in diets with 0.1% NRYE quadratically increased ADG ($P = 0.069$) and G:F ($P = 0.012$; Table 2), and in phase 2 showing that increasing AGP level in diets with 0.1% NRYE, quadratically increased ATTD of DM ($P = 0.001$), CP ($P = 0.001$) and GE ($P = 0.017$; Table 3). In both cases, the highest values of the measured parameters were associated with 25 and 50% supplementation of the recommended AGP dosage implying that

Table 2
Growth performance of pigs fed diets without or with 0.1% of a nucleotide-rich yeast extract (NRYE) with graded levels of antimicrobial growth promoters (AGP), phase 1 is from d 1 to 14, phase 2 is from d 15 to 28.

Diet	NRYE, %	AGP[1], %	ADFI, g/d			ADG, g/d			G:F, g/g		
			Phase 1	Phase 2	Overall	Phase 1	Phase 2	Overall	Phase 1	Phase 2	Overall
1	0	0	369	701	535	282	442	362	0.77	0.64	0.67
2	0.1	0	416	685	551	314	560	437	0.76	0.83	0.80
3	0.1	25	415	662	538	328	491	410	0.79	0.77	0.77
4	0.1	50	416	716	566	345	408	376	0.83	0.58	0.67
5	0.1	75	416	691	554	310	469	389	0.75	0.69	0.71
6	0.1	100	395	670	533	278	412	345	0.70	0.62	0.65
Pooled SEM			22.8	38.2	21.4	19.7	32.6	21.2	0.028	0.06	0.04
Contrasts[2]											
Linear			NS	NS	NS	NS	0.002	0.002	NS	0.007	0.006
Quadratic			NS	NS	NS	0.069	NS	NS	0.012	NS	NS
Diet 2 vs. 1			NS	NS	NS	NS	0.017	0.019	NS	0.035	0.040
Diet 2 vs. 6			NS	NS	NS	NS	0.004	0.005	NS	0.021	0.016

ADFI = average daily feed intake; ADG = average daily gain; G:F = gain to feed ratio; SEM = standard error of mean; NS = not significant.

[1] AGP, 100% = 55 mg of Aueromycin (chlortetracycline) + 31.2 mg of Tiamulin per kg of diet.

[2] Linear and quadratic contrasts were determined for treatments supplemented with 0.1% NRYE; preplanned contrasts were determined between Diet 2 vs. 1 and Diet 2 vs. 6.

Table 3
Apparent total tract digestibility (ATTD) of dry matter (DM), crude protein (CP) and gross energy (GE) of pigs fed diets without or with 0.1% of a nucleotide-rich yeast extract (NRYE) with graded levels of antimicrobial growth promoters (AGP), phase 1 is from d 1 to 14, phase 2 is from d 15 to 28.

Diet	NRYE, %	AGP[1], %	DM, %		CP, %		GE, %	
			Phase 1	Phase 2	Phase 1	Phase 2	Phase 1	Phase 2
1	0	0	77.2	77.7	70.0	72.6	74.8	76.7
2	0.1	0	73.5	76.3	66.7	70.9	71.7	74.9
3	0.1	25	74.0	78.9	62.8	76.5	71.6	77.4
4	0.1	50	77.0	78.1	65.7	74.0	75.3	76.9
5	0.1	75	71.7	77.3	61.7	73.3	69.3	75.8
6	0.1	100	73.8	75.8	65.4	70.5	71.6	74.5
Pooled SEM			1.52	0.83	2.15	1.17	1.73	0.92
Contrast[2]								
Linear			NS	NS	NS	NS	NS	NS
Quadratic			NS	0.001	NS	0.003	NS	0.017
Diet 2 vs. 1			NS	NS	NS	NS	NS	NS
Diet 2 vs. 6			NS	NS	NS	NS	NS	NS

SEM = standard error of mean; NS = not significant.

[1] 100% of recommended AGP dosage = 55 mg of Aueromycin (chlortetracycline) and 31.2 mg of Tiamulin per kg of diet.

[2] Linear and quadratic contrasts were determined for treatments supplemented with 0.1% NRYE; preplanned contrasts were determined between Diet 2 vs. 1 and Diet 2 vs. 6.

supplementing 0.1% NRYE can replace in-feed AGP dosage by 75% without suppressing performance and nutrient utilization. The effect of supplementing NRYE and AGP on the ATTD of CP, DM and GE was investigated because transition from milk to solid feed imparts stress to the development and function of the immature gut (van Beers-Schreurs et al., 1998), which may result in slow maturation of the gut mucosa and consequently insufficient secretion of digestive enzymes. Together, these factors may result in poor digestion and absorption of nutrients thus contributing to the growth depression observed after weaning.

It is worth noting that the NRYE product used in this study also contained yeast cell wall polysaccharides (21.6%) which may help in the modulation of mucosal immunity (Kogan and Kocher, 2007). Therefore, the beneficial effects of the NRYE may be multi-faceted and cannot be exclusively attributed to the effect of nucleotides present in the product (Sauer et al., 2012).

4. Conclusions

Dietary NRYE supplementation improved growth performance but increasing AGP level reduced the beneficial effect of NRYE. The NRYE can be supplemented alone or with 25% of the recommended AGP dosage without affecting growth performance and nutrient utilization in weaned pigs. However, further studies are needed to substantiate the importance of supplemental NRYE in improving piglet growth performance post-weaning and their effectiveness towards feeding AGP-free diets. Overall, these results show the importance of supplemental NRYE in improving piglet growth performance post-weaning and may be beneficial to producers and nutritionists desiring to adapt AGP-free feeding programs.

Conflict of interest

The authors declare that there are no conflicts of interest.

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