EFFECTS OF INULIN AND A PROBIOTIC MIXTURE ON NUTRIENT DIGESTIBILITY AND NITROGEN BALANCE IN PIGLETS

EFEITO DA INULINA E DE PROBIÓTICOS SOBRE A DIGESTIBILIDADE DOS NUTRIENTES E BALANÇO DO NITROGÊNIO EM LEITÕES

Rodrigues, M.[1]*; Pozza, P.C.[2]; Pozza, M.S.S.[3A]; Possamai, M.[3B]; Bruno, L.D.G.[3C]; Richart, E.[3D]; Wochner, M.O.[3E] and Pereira Júnior, J.M.[3F]

[1]Universidade Estadual Paulista. Faculdade de Medicina Veterinária e Zootecnia. Departamento de Produção Animal. Botucatu, SP. Brasil. *mayara_rodriguez@yahoo.com.br

[2]Universidade Estadual de Maringá. Departamento de Zootecnia. Maringá, PR. Brasil. pcpozza@yahoo.com.br

[3]Universidade Estadual do Oeste do Paraná. Marechal Cândido Rondon, PR. Brasil. [A]magaspozza@hotmail.com; [B]dm_maikel@hotmail.com; [C]ldgbruno@gmail.com; [D]edson_richart@hotmail.com; [E]manomw@hotmail.com; [F]joao_morais@zootecnista.com.br

ADDITIONAL KEYWORDS

Bifidobacterium bifidum. Digestibility. Oligosaccharides.

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SUMMARY

This study aimed to evaluate different inulin and probiotic levels as supplement in diets for piglets on nutrient digestibility and nitrogen balance. Twenty four crossbred barrows (Pietráin x Landrace x Large White), with initial average weight of 18.00 ± 0.38 kg, were individually housed in metabolic cages. The experimental design was a completely randomized block, in a 2 x 3 factorial scheme (probiotic levels: 0.30 and 0.60 %; inulin levels: 0.00, 0.25 and 0.50 %), with four replications. The probiotic used was a mix of *Lactobacillus acidophillus, Streptococcus faecium* and *Bifidobacterium bifidum*. The inulin was the prebiotic used in this study, characterized as an indigestible carbohydrate formed by fructooligosaccharides. Inulin levels provided a quadratic effect (p<0.05) on the digestibility coefficients of dry matter, organic matter and ether extract, and the better responses were obtained supplementing 0.194, 0.185 and 0.188 %. Quadratic effects were observed for the nitrogen excreted in feces, total nitrogen excretion, nitrogen efficiency use and nitrogen digestibility. The inulin levels of 0.194 and 0.216 %, in piglet diets, were the better for dry matter digestibility and total nitrogen excretion, respectively.

RESUMO

O objetivo deste trabalho foi avaliar o efeito de níveis de inulina e probióticos em rações para leitões sobre a digestibilidade dos nutrientes e balanço do nitrogênio. Foram utilizados 24 suínos mestiços (Pietráin x Landrace x Large White), machos castrados, com peso médio inicial de 18,00 ± 0,38 kg, alojados individualmente em gaiolas de metabolismo. O delineamento experimental utilizado foi o de blocos ao acaso em esquema fatorial 2 x 3, representado por dois níveis de probiótico (0,30 e 0,60 %) e três níveis de inulina (0,00; 0,25 e 0,50 %), com quatro repetições. O probiótico utilizado foi composto por *Lactobacillus acidophillus, Streptococcus faecium* e *Bifidobacterium bifidum*. A inulina foi o prebiótico utilizado, caracterizada como um carboidrato não digerível composto por frutoligossacarídeos. A inulina proporcionou efeito quadrático (p<0,05) nos coeficientes de digestibilidade da matéria seca, matéria orgânica e extrato etéreo, e as melhores respostas foram obtidas com a suplementação de 0,194; 0,185 e 0,188 %. Efeitos quadráticos foram observados para o nitrogênio excretado nas fezes, excreção total de nitrogênio, eficiência de uso do nitrogênio e digestibilidade do nitrogênio. Os níveis de 0,194 e 0,216 % de inulina,

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na ração de leitões, foram os melhores para a digestibilidade da matéria seca e excreção total de nitrogênio, respectivamente.

INTRODUCTION

Alternative ingredients to substitute growth promoters in swine diets are studied and developed based on new concepts of food safety, such as prebiotics and probiotics that, when used together and with specificity, establishes the symbiotic concept and can be used in swine diets.

Probiotics are live cultures of organisms supplemented in pig diets that can beneficially affect the host animal by improving the microbial balance in the gut (Fuller, 1989), and may influence digestive process by increasing the population of beneficial microorganisms and activity of microbial enzymes, as well as improving nutrient digestibility and use.

Prebiotics are defined as a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon (Gibson and Roberfroid, 1995). These compounds are indigestible oligosaccharides (Delzenne, 2003) not necessarily grouped by the structure, but according to their capacity to promote growth of certain beneficial bacteria (probiotics) in the gut (Kelly, 2008). Inulin is known as a prebiotic and consists of a mixture of fructan chains with a polymerizing degree varying from 2 to 60 (Roberfroid, 1993), is resistant to digestive enzymes, due to its glycoside β bonds (2→1), and is believed to increase growth of beneficial bacteria (Zentek *et al.*, 2003).

The use of prebiotics, probiotics or its combination as a simbiotic is an alternative to replace growth promoters without impairing the swine performance (Chiquieri *et al.*, 2006). Using a symbiotic in diets for weaned piglets, Shim *et al.* (2005) reported an increased villous height in the small gut, which is an indicative of improved nutrient absorption. Pierce *et al.* (2005) observed an improvement in gross energy digestibility in piglet diets containing 1.50 % of inulin and a low lactose level.

Prebiotics may also reduce the nitrogen excretion to the environment. Besides, Lynch *et al.* (2007) reported that supplementing inulin in piglet diets may favor the bifidobacteria growing in the cecum, increase fecal nitrogen content and reduce the nitrogen amount in urine.

Therefore, the prebiotic's effect on the nitrogen contained in the diet occurs due to fermentation in the large gut, which induces changes in urine nitrogen excreted as urea (Zervas and Zijlstra, 2002). According to Nahm (2003), the result is a decrease in ammonia emision during waste storage and field application.

This study aimed to evaluate the effects of inulin and probiotic on nutrient digestibility and nitrogen balance in piglets.

MATERIAL AND METHODS

This study was carried out in the metabolism room, situated in the experimental of the Universidade Estadual do Oeste do Paraná, Brazil. The experimental procedures were in agreement with the Animal Welfare Committee of this Institution.

Twenty four crossbred barrows (Pietráin x Landrace x Large White), averaging 18.00 ± 0.38 kg of initial weight, were individually allotted and distributed in a completely randomized blocks design, in a 2 x 3 factorial scheme (probiotic levels – 0.30 and 0.60 %; inulin levels – 0.00, 0.25 and 0.50 %), with four replicates.

The probiotic used was a mix of *Lactobacillus acidophillus, Streptococcus faecium* and *Bifidobacterium bifidum*. The inulin was the prebiotic used in this study, characterized as an indigestible carbohydrate formed by fructooligosaccharides.

Experimental diets **(table I)** were formulated to achieve the nutritional requirements proposed by Rostagno *et al.* (2005). Barrows

Table I. *Centesimal, chemical and energetic composition of experimental diets.* (Composição centesimal, química e energética das rações experimentais).

Probiotic		0.30			0.60	
Inulin	0.00	0.25	0.50	0.00	0.25	0.50
Corn	56.66	56.66	56.66	56.66	56.66	56.66
Soybean meal	21.00	21.00	21.00	21.00	21.00	21.00
Dried whey	6.00	6.00	6.00	6.00	6.00	6.00
Fish meal	4.00	4.00	4.00	4.00	4.00	4.00
Corn gluten meal	3.00	3.00	3.00	3.00	3.00	3.00
Soybean oil	2.70	2.70	2.70	2.70	2.70	2.70
Sugar	2.00	2.00	2.00	2.00	2.00	2.00
Common salt	0.34	0.34	0.34	0.34	0.34	0.34
Limestone	0.31	0.31	0.31	0.31	0.31	0.31
Dicalcium phosphate	1.44	1.44	1.44	1.44	1.44	1.44
Choline chloride	0.20	0.20	0.20	0.20	0.20	0.20
L-lysine hydrochloride	0.52	0.52	0.52	0.52	0.52	0.52
DL-methionine	0.17	0.17	0.17	0.17	0.17	0.17
L-threonine	0.23	0.23	0.23	0.23	0.23	0.23
L-tryptophan	0.05	0.05	0.05	0.05	0.05	0.05
Antioxidant[1]	0.01	0.01	0.01	0.01	0.01	0.01
Organic acids[2]	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin supplement[3]	0.12	0.12	0.12	0.12	0.12	0.12
Mineral supplement[4]	0.05	0.05	0.05	0.05	0.05	0.05
Probiotic[5]	0.20	0.20	0.20	0.40	0.40	0.40
Bifidobacteria[6]	0.10	0.10	0.10	0.20	0.20	0.20
Inulin[7]	0.00	0.25	0.50	0.00	0.25	0.50
Inert	0.80	0.55	0.30	0.50	0.25	0.00
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated composition						
Metabolizable energy (kcal kg^{-1})	3,325	3,325	3,325	3,325	3,325	3,325
Crude protein (%)	19.67	19.67	19.67	19.67	19.67	19.67
Calcium (%)	0.83	0.83	0.83	0.83	0.83	0.83
Available phosphorus (%)	0.45	0.45	0.45	0.45	0.45	0.45
Sodium (%)	0.23	0.23	0.23	0.23	0.23	0.23
Potassium (%)	0.73	0.73	0.73	0.73	0.73	0.73
Chlorine (%)	0.37	0.37	0.37	0.37	0.37	0.37
Digestible lysine (%)	1.33	1.33	1.33	1.33	1.33	1.33
Digestible methionine + cystine (%)	0.75	0.75	0.75	0.75	0.75	0.75
Digestible methionine (%)	0.47	0.47	0.47	0.47	0.47	0.47
Digestible threonine (%)	0.85	0.85	0.85	0.85	0.85	0.85
Digestible tryptophan (%)	0.23	0.23	0.23	0.23	0.23	0.23

[1]BHT; [2]Propionic, formic and lactic acids; [3]Vitamin A (8 000 000 IU); Vitamin D3 (1 200 000 IU); Vitamin E (20 000 mg); Vitamin K3 (2500 mg); Thiamine (1000 mg); Riboflavine (4000 mg); Vitamin B12 (20 mg), Niacin (25 000 mg); Pantothenic acid (10 000 mg); Biotin (50 g); Folic acid (600 mg); Vitamin C (50 000 mg), Antioxidant (125 mg); [4]Zinc (80 000 mg); Fe (70 000 mg); Mn (40 000 mg); Cu (20 000 mg); I (800 mg); Co (500 mg); Se (500 mg), QSAD vehicle (1000 g); [5]*Lactobacillus acidophillus, Streptococcus faecium*; [6]*Bifidobacterium bifidum*; [7]Inulin (96 %).

were fed twice a day (06 a.m. and 06 p.m.) and after each meal the water was freely offered. The individual feed intake was based on the metabolic weight ($BW^{0.75}$), established during the adjustment period.

The metabolism assay used a seven day adjustment period, followed by a five day period of total collection of feces and urine. Barrows were housed in stainless-steel metabolism cages (Pekas, 1968), equipped with feeders and compartments which allowed separated collection of feces and urine.

Ferric oxide (Fe_2O_3) was used to indicate the beginning and the end of fecal collection period. Feces were collected twice a day, placed into plastic bags and stored (-18 °C). Urine was collected continuously over the 5-day collection period into a plastic recipient, containing 20 mL of 1:1 chloridic acid (HCl), and a 5 % aliquot was placed into glass containers, identified and stored (3 °C).

Prior to laboratory analysis feces were thawed, mixed, dried (55 ºC) and ground through a 1 mm screen. Dry matter (DM), nitrogen (N), ether extract (EE) and mineral matter (MM) were analysed in diets and feces, and N were determined in urine, as described by Silva and Queiroz (2002).

The coefficients of apparent digestibility of dry matter (DCDM), ether extract (DCEE), mineral matter (DCMM) and organic matter (DCOM) were determined according to Matterson *et al.* (1965). Nitrogen intake and total N excretion were determined to obtain the N balance.

Data was submitted to variance analysis through the statistical software SAEG-System for Statistical and Genetic Analyses, developed by the Federal University of Viçosa (UFV, 1999). Data was analyzed in a factorial scheme (probiotc x inulin), and were applied the regression procedures in case of significance.

RESULTS AND DISCUSSION

Significant interactions (inulin x probiotic) were not observed ($p>0.05$) for nutrient digestibility **(table II)**. The probiotic

Table II. *Apparent digestibility in diets for piglets supplemented with inulin and probiotics.* (Digestibilidade de rações para leitões contendo inulina e probióticos).

	Levels						Means								
Probiotic	0.3			0.6			Probiotic		Inulin			CV	p		
Inulin	0	0.25	0.5	0	0.25	0.5	0.3	0.6	0	0.25	0.5	(%)	Prob	Inu	p x I
DCDM	86.7	87.8	84.9	86.8	87.8	85.4	86.5	86.7	86.8	87.8	85.2	2.29	NS	0.044	NS
DIGDM	79.2	80.2	77.5	80.3	80.6	78.0	79.0	79.6	79.8	80.4	77.8	-	-	-	-
DCOM	88.7	89.6	86.4	88.5	89.3	86.9	88.2	88.2	88.6	89.5	86.7	2.09	NS	0.021	NS
DIGOM	75.5	76.8	73.7	75.9	77.1	74.9	75.3	76.0	75.7	77.0	74.3	-	-	-	-
DCMM	45.8	48.7	46.0	46.2	51.1	48.3	46.8	48.6	46.0	49.9	47.2	7.46	NS	NS	NS
DIGMM	2.0	2.5	2.4	2.4	2.6	2.5	2.3	2.5	2.2	2.6	2.5	-	-	-	-
DCEE	82.7	86.5	77.3	80.9	82.2	76.1	82.1	79.7	81.8	84.4	76.7	4.11	NS	0.001	NS
DIGEE	4.9	5.2	4.6	5.0	4.9	4.6	4.9	4.8	5.0	5.1	4.6	-	-	-	-

NS: non significant; DC= digestibility coefficient; DIG= digestible; DM= dry matter; OM= organic matter; MM= mineral matter; EE= ether extract.

studied did not affected ($p>0.05$) the nutrient digestibility, but inulin levels provided a quadratic effect ($p<0.05$) on the digestibility coefficients of dry matter, organic matter and ether extract, and the better responses were obtained supplementing 0.194, 0.185 and 0.188 % of inulin **(figure 1)**, respectively, because above these levels the digestibility coefficients decreased, and Hedemann *et al.* (2001) reported that a decrease in the nutrient digestibility is not desired in piglet diets.

Such enhancements in nutrient digestibility were previously observed in piglet diets with low-lactose supplemented with inulin (Pierce *et al.*, 2005). The decrease in digestibility coefficients, observed for the high inulin levels **(figure 1)**, may be related to a reduction in the number of goblet cells in the jejunum, as observed in weanling pigs fed diets with 0.40 % inulin (Mair *et al.*, 2010), and Pickler *et al.* (2012) reported that the glycoproteins produced by goblet cells participate in the nutrient absorption.

However, higher inulin levels could demonstrate good results, depending on the lactose level used in the experimental diets. Lynch *et al.* (2009) observed an increase in digestibility of dry matter, organic matter and mineral matter in piglet diets containing lactose (5.00 %) and inulin (1.50 %), but this response were not observed with high lactose level (23.00 %). On the other hand, Lynch *et al.* (2007) evaluating

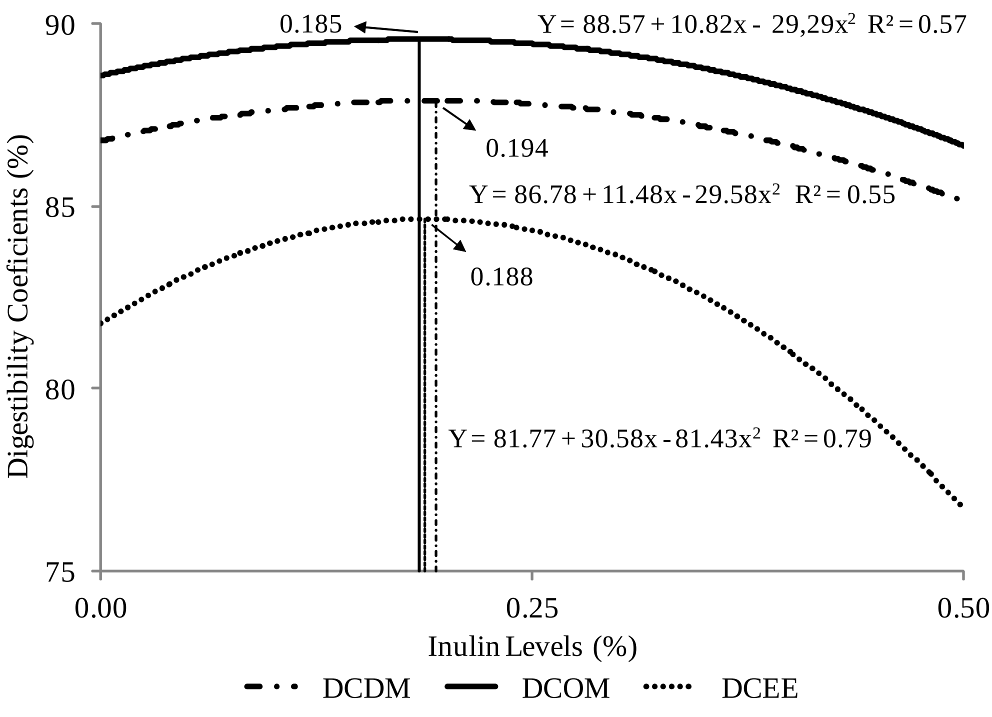

***Figure 1 .** Digestibility coefficients of dry matter (DCDM), organic matter (DCOM) and ether extract (DCEE) affected by inulin levels in piglet diets.* (Coeficientes de digestibilidade da matéria seca (DCDM), matéria orgânica (DCOM) e do extrato etéreo (DCEE) em função de níveis de inulina em rações para leitões).

diets with low and high levels of crude protein, supplemented with 0.00 and 1.25 % inulin, did not observe any effects of inulin in the digestibility of dry matter and organic matter for finishing pigs.

Evaluating the inclusion of *Enterococcus faecium* and inulin (2.00 %) in piglet diets Böhmer *et al.* (2005) reported a positive effect of inulin, and inulin with *E. faecium*, compared to the control diet and containing just *E. faecium*.

For the nitrogen balance there were no significant (p>0.05) interactive effects of inulin and probiotic **(table III)**. The nitrogen intake was similar for the evaluated treatments because experimental diets were isonitrogenous and animals were fed based on metabolic weight ($BW^{0.75}$).

The probiotic used did not affect (p>0.05) the nitrogen balance variables, but a quadratic effect (p<0.01) was observed for the nitrogen excreted in feces **(figure 2)**. According to Vanhoof and De Schrijver (1996), increased nitrogen excretion may be resulted from higher microbial activity in the large intestine and thus higher nitrogen incorporation in microbial protein excreted in feces. Hansen *et al.* (2010) observed higher bacterial activity in the upper colon in pigs fed a diet with 8.00 % inulin. *In vitro* studies to evaluate different indigestible carbohydrates, and bacterial isolates, demonstrated that inulin and starch increased bacterial nitrogen fixing, compared to cellulose, xylan and pectin (Bindelle *et al.*, 2007a; 2007b).

In the current study, the increase in inulin levels, higher than 0.170 %, increased the nitrogen excreted in feces. This inulin level is lower than 1,25 % used by Lynch *et al.* (2007) which observed higher amounts of nitrogen in swine feces when the animals were supplemented with 1.25 % inulin in the diet. However the nitrogen excreted in urine was not affected by the inulin levels. In the same way, Halas *et al.* (2010a) observed an increase in fecal nitrogen, but the urine nitrogen decreased in weanling pigs that

Table III. *Nitrogen balance in piglets submitted to diets with containing inulin and probiotics.* (Balanço de nitrogênio em leitões submetidos à dietas contendo inulina e probióticos).

	Levels						Means								
Probiotic	0.3			0.6			Probiotic		Inulin			CV	p		
Inulin	0	0.25	0.5	0	0.25	0.5	0.3	0.6	0	0.25	0.5	(%)	Prob	Inu	p x I
NI (g/d)	55.0	55.0	54.1	56.3	58.9	54.5	54.7	56.6	55.7	57.0	54.3	13.03	NS	NS	NS
NEF (g/d)	4.4	4.1	5.6	4.5	4.2	5.5	4.7	4.7	4.5	4.2	5.6	11.48	NS	0.009	NS
NEUr (g/d)	9.1	9.1	10.1	9.7	6.5	9.3	9.4	8.5	9.4	7.8	9.7	18.36	NS	NS	NS
TNE (g/d)	13.5	13.2	15.7	14.2	10.7	14.8	14.1	13.2	13.9	12.0	15.3	12.36	NS	0.038	NS
NR (g/d)	41.5	41.8	38.4	42.1	48.2	39.8	40.6	43.4	41.8	45.0	39.1	13.49	NS	NS	NS
NEU (%)	75.5	76.4	70.1	75.6	81.7	72.4	74.0	76.6	75.6	79.1	71.3	5.01	NS	0.009	NS
ND (%)	91.9	92.4	89.7	91.8	92.8	89.7	91.3	91.4	91.9	92.6	89.7	1.67	NS	0.008	NS

NS: non significant; NI: nitrogen intake; NEF: nitrogen excreted in feces; NEUr: nitrogen excreted in urine; TNE: total nitrogen excretion; NR: nitrogen retention; NEU: nitrogen efficiency use; ND: nitrogen digestibility.

received 4.00 or 8.00 % of inulin in diets, being a desired effect for the environment, since it decreases the ammonia emission due to fecal nitrogen be consisted primarily by bacterial protein, which is less susceptible to decomposition than the urea present in the urine, because urea is easily converted into ammonia and carbon dioxide by bacterial urease (Mroz *et al*., 2000).

However, the levels of inulin used in these studies were higher than the ones used in the current research (0.00 – 0.50 %), and using a low-inulin level (0.40 %) in diets for growing pigs Loh *et al.* (2010) reported no effect on the nitrogen excretion, but the inulin tended to shift nitrogen excretion from urine to feces.

The inverse response obtained for nitrogen excreted in feces and nitrogen digestibility (**figures 2** and **3**) also was found by Lynch *et al.* (2007) using more than 1.25 % inulin in diets for finishing pigs. Nevertheless, Lynch *et al.* (2009) observed an increased nitrogen digestibility in piglets supplemented with 1.50 % inulin in a diet containing 5.00 % lactose. According to these authors, inulin may increase the population of lactic bacterias, like bifidobacteria. Their capacity to produce a wide variety of depolymerases and glycosidases, which acts over polysaccharides, may improve digestion. Even so, Vanhoof and De Schrijver (1996) and Halas *et al.* (2010b) did not report any differences on nitrogen digestibility after supplementing 6.00 and 8.00 % of inulin in piglet diets.

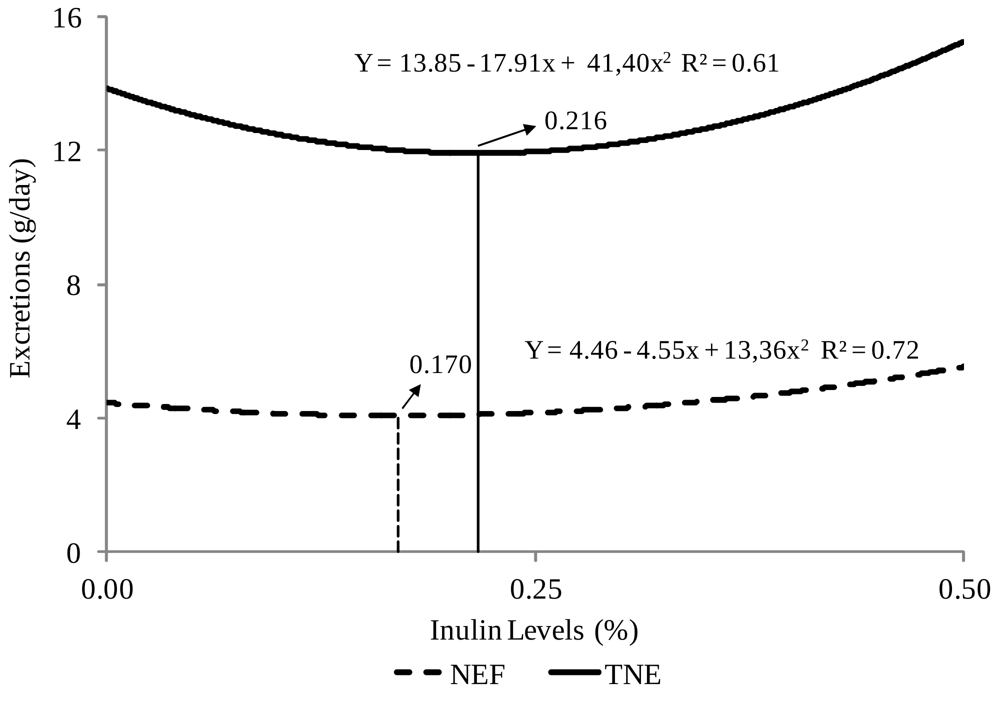

***Figure 2.** Effect of inulin levels in piglet diets on nitrogen excreted in feces (NEF) and total nitrogen excretion (TNE).* (Efeito de níveis de inulina na ração sobre o nitrogênio excretado nas fezes (NEF) e excreção total de nitrogênio (TNE) em leitões).

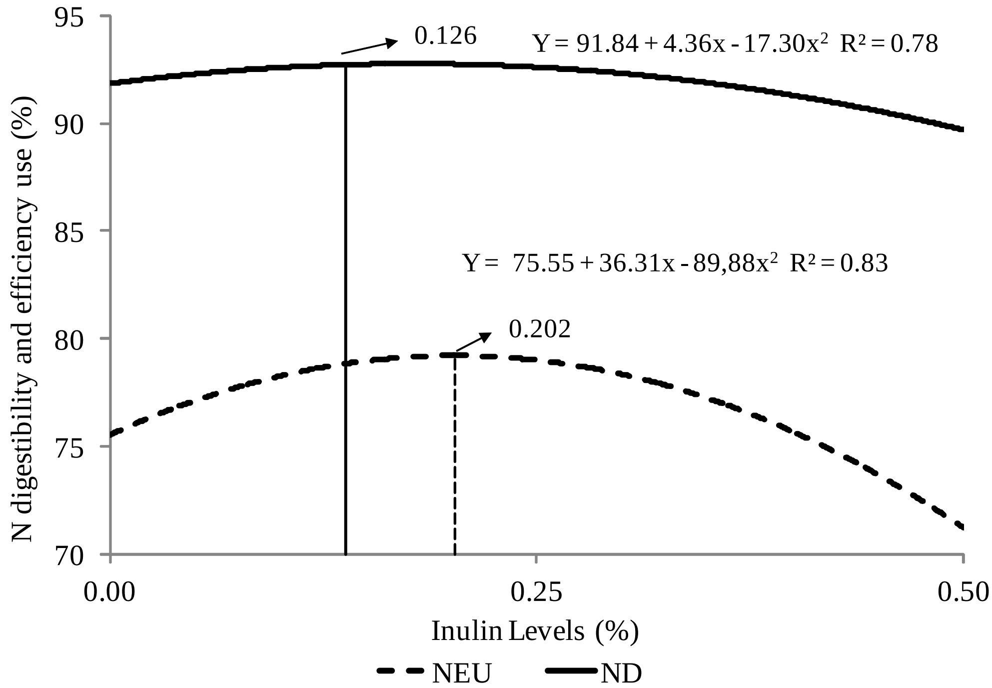

***Figure 3 .** Effect of inulin levels in piglet diets on nitrogen efficiency use (NEU) and nitrogen digestibility (ND).* (Efeito de níveis de inulina na ração sobre a eficiência de uso do nitrogênio (NEU) e digestibilidade do nitrogênio (ND) em leitões).

The results obtained for nitrogen excreted in feces, and urine, influenced the total nitrogen excretion ($p<0.05$), showing the better excretion at a level of 0.216 % inulin **(figure 2)**. Although total nitrogen excretion was affected by the inulin and nitrogen intake remained constant, there was no difference in nitrogen retention **(table III)**. This effect was also evidenced by a quadratic effect observed for nitrogen efficiency use **(figure 3)**, which is the percentage of nitrogen retained, showing that the supplementation of 0.202 % inulin provided the better efficiency, that is proximate of 0.216 % found for total nitrogen excretion. Lynch *et al.* (2007) and Vanhoof and De Schrijver (1996) did not obtain significant response for the efficiency of nitrogen utilization according to inulin levels studied. Likewise, Mountzouris *et al.* (2006) evaluated two indigestible carbohydrates (oligofructose and transgalactoligosaccharides) as supplement in piglet and did not observe significant effects on the efficiency of nitrogen use.

CONCLUSIONS

The inulin levels of 0.194 and 0.216 %, in piglet diets, were the most effectives for dry matter digestibility and total nitrogen excretion, respectively.

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