The use of nucleotides, vitamins and functional amino acids to enhance the structure of the small intestine and circulating measures of immune function in the post-weaned piglet

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Abstract

Ninety individually-housed castrated pigs (Large White × Landrace × Duroc mixed crossbred, n=18) were used in a randomized block experiment to determine the effect of yeast protein concentrate (YPC) or it's major active components, nucleotides (NCL), inositol (INS), and glutamate (GLU), on pig performance, indices of gut structure and circulating measures of immune function. Daily gain and feed intake were not affected by diet, however pigs fed the YPC diet had a lower feed conversion ratio compared to those fed the control (CON), INS and NCL diets (P=0.028) in the feeding period. Villous height in the duodenum was increased in pigs that received the YPC diet compared to the CON and INS diets (P=0.029). In addition, immunoglobulin G levels were increased in pigs that received the INS and GLU diets compared to the CON and NCL diet on day 21 (P=0.034). These data suggest that although the effect was limited on the duodenal villous structure, pigs fed the YPC diet showed an improved duodenal villous height and the positive effect of YPC is most likely attributable to glutamate and nucleotides in the YPC.

Keywords:

Growth performance; Inositol, Glutamate, Nucleotides, Pigs

1. Introduction

The period immediately after weaning can be stressful for the pig because of the multitude of changes that occur simultaneously at that time. As a consequence, the growth rate and feed conversion efficiency of pigs following weaning is reduced relative to that observed during lactation and well below the pigs' genetic potential (King and Pluske, 2003). This growth check at weaning can adversely affect pigs' long-term performance (Pluske et al., 1997; Dirkzwager et al., 2005).

A key contributor to the growth check after weaning is the structural and functional changes in the small intestine, such as villous atrophy and crypt hyperplasia. These changes are usually associated with a decrease in digestive and absorptive capacity (Pluske et al., 1997). The importance of food intake and dietary exogenous growth factors as mediators of intestinal structure and function after weaning has been widely reviewed and has been shown to reduce the impact of the post-weaning growth check (Cranwell, 1995; Pluske et al., 1997; Lalles et al., 2007). For example, the provision of oral glutamine to the young piglet has been shown to support mucosal growth thereby maintaining villous integrity and the structure and function of the small intestine (Wu et al., 2007). Glutamate also has a distinct flavour which is thought to enhance palatability (Diehl, 2004) resulting in improved feed intake. Another nutrient that has been suggested as having a role in reducing the post-weaning growth check is inositol, that is a fundamental component of cell membranes and is necessary for the proper function of the small intestinal mucosal cells (Kroes et al., 1973).

Numerous human and animal studies suggest that dietary nucleotides play a role in the development of the gastrointestinal and immune systems (Carver, 2003; Dirkzwager et al., 2005; Lee et al., 2007), and that collectively they reduce the growth check that occurs in almost all baby mammals after weaning (Rutz et al., 2006). The concentration of nucleotides in sow's milk is many times greater than that in a high quality creep feed (Mateo et al., 2004), suggesting that if these levels were increased in solid feed offered to pigs then it might help piglets with the transition at weaning.

While nucleotides, inositol and glutamate may all play a role in reducing the growth check of piglets at weaning, it is also possible that their supply in increased concentrations fed simultaneously may have additive effects. Therefore, the aim of this experiment was to determine the effect of the addition of nucleotides, inositol or glutamate to creep and weaner diets, either separately or in combination, on indices of gut structure, circulating immune function and lifetime performance.

2. Materials and methods

2.1 Experimental design

Ninety Large White × Landrace × Duroc mixed crossbred (PIC) and surgically castrated male pigs were used in a randomized block experiment with 18 individually-housed pigs per treatment as the experimental unit. The dietary treatments were: 1) control (CON); 2) inositol (INS); 3) glutamate (GLU); 4) nucleotides (NCL) and 5) yeast protein concentrate (YPC) containing the same amounts of inositol, glutamate, and nucleotides to the INS, GLU and NCL diets. The protocol used in this experiment

conformed to all regulations of the Western Australian Department of Agriculture and Food Animal Ethics Committee (AEC Activity No. 3-08-18) concerning the health and care of experimental animals.

2.2 Animals and diets

The pigs were obtained at weaning (approximately 3 weeks of age) from a commercial supplier and transported to the research station where they were randomly allocated to treatment after stratification on live weight. Pigs were blocked based on live weight and area of the room. The pigs weighed (mean ± SE) 6.14 ± 0.10 kg on arrival. They were housed in individual wire-mesh pens (0.6 × 0.6 m) in an environmentally controlled facility. Ambient temperature was maintained at 30°C for the first week after weaning, and then dropped by 1°C each week thereafter. At 6 weeks of age the pen area per pig was doubled and then at 8 weeks of age the pigs were moved to a naturally ventilated grower-finisher shed where they were again housed in individual pens (1.8 × 0.9 m) until slaughter. All pigs had *ad libitum* access to feed and water.

The experimental diets were fed as a mash for 21 days after weaning, with a first stage weaner diet (weaner 1) being fed for the first 7 days followed by a second stage weaner diet (weaner 2) that was fed for the next 14 days. The weaner 1 diets and weaner 2 diets were formulated to contain 15.2 MJ DE/kg and 0.9 g available lysine/MJ DE and 14.9 MJ DE/kg and 0.85 g available lysine/MJ DE, respectively. The composition of these diets and calculated nutrient composition are given in Table 1. Diets were not analysed for any nutrient. For weaner 1 diets containing inositol,

glutamate and nucleotides, the additives were included at 0.12 g, 2.7 g and 2 g/kg, respectively. For Weaner 2 diets, the doses used were 0.06 g, 1.35 g and 1 g/kg, respectively. The inclusion levels of inositol, glutamate and nucleotides in weaner 1 and weaner 2 diets were calculated based on the respective composition in the YPC product. For the YPC treatment, the diet was re-formulated to take account of the inclusion level and amino acid composition of the product. The product was included at 40 g/kg and 20 g/kg in the weaner 1 and weaner 2 diets, respectively. The YPC (Nupro, Alltech Inc., Kentucky, USA) is cell contents of the *Saccharomyces cervisiae* yeast containing 3.6 g inositol, 67.6 g glutamate and 70 g nucleotides per kg product. The Ascogen was purified nucleotides obtained from the *Saccharomyces cervisiae* yeast. The additives in the YPC and NCL diets were added at the recommended product inclusion rate, while the levels of INS and GLU were included at the same rate as present in the YPC diet. At the end of the experimental diet phase (i.e. 6 weeks of age), all pigs were fed the same commercial diets until slaughter.

When pigs were approximately 20 weeks of age (an average of 101 kg LW), all feed was removed from the feeders in the evening before slaughter, and the following morning all pigs were transported to a commercial abattoir. Pigs were stunned using a CO_2, dip-lift stunner set at 85% CO_2 for 1.8 minutes in a commercial abattoir (Butina, Denmark, WA, Australia). Exsanguination, scalding, dehairing and evisceration were performed using standard commercial procedures.

2.3 Growth performance, blood sampling and analysis, and small intestinal histology

The pigs were weighed weekly from weaning until slaughter. Voluntary feed intake (FI) was recorded on a weekly basis and feed conversion ratio (FCR) was calculated by dividing the total weight of feed eaten by the liveweight gain in the same period. Carcass weight and backfat depth at the P2 site were determined on the hot carcass at 45 minutes after slaughter. Backfat depth at the P2 site was measured using the PorkScan Carcass Classification System (Pork Scan Pty Ltd, Brisbane, Australia).

After the initial 7-day feeding period, 8 piglets representing the treatment group from each treatment were selected by identifying the median weight and then randomly selecting 4 pigs from above the median and 4 pigs below the median. The selected piglets were then blood sampled via jugular vein puncture. Approximately 10 mL of blood was collected into serum vacutainer tubes spray-coated with silica (BD Vacutainer Systems, Plymouth, UK). Blood from the same 8 piglets was also collected at the conclusion of the 3-week feeding period. The blood samples were centrifuged at 2000 × g for 10 min at 5°C and the serum was stored at -20°C until analysed. Haptoglobin in serum was quantified using a spectrophotometric method with a commercial kit (Phase^{TM} Haptoglobin Assay, Tridelta Limited, Ireland). The assay was performed on an automated analyser according to the manufacturer's instructions (Olympus AU400; Olympus UK Ltd, Hertfordshire, United Kingdom). Immunoglobulins G (IgG) , A (IgA) and M (IgM) were determined by ELISA (Bethyl Laboratories Inc., Texas, United States).

After the 3-week feeding period, the same 8 piglets that were blood sampled were euthanased and a mid-line incision was performed to locate the gastrointestinal

tract (GIT). The entire GIT was removed and 2 cm intestinal samples of the duodenum, jejunum and ileum were collected. The samples were placed in a 70% ethanol solution for subsequent storage. The tissue samples were processed onto paraffin wax blocks and cut into 5 μm thick cross sections using a microtome. The sections were then mounted onto polylysine coated slides and stained with hematoxylin and eosin. The villous height and crypt depth were determined using a binocular light microscope at a magnification of x10. The crypt depth was measured from the junction with the villous to the base of the crypt. Villous height was measured from the crypt orifice to the tip of the villous. Approximately 10 measurements were taken on each slide and the mean villous height and crypt depth determined. The villous height to crypt depth ratio was also calculated.

2.4 Statistics

One-way analysis of variance (ANOVA) with the Genstat 11 program (VSN International Ltd., Hemel Hempstead, UK) was used to analyse the main effect of diet. Pig was considered the experimental unit and position within the shed was used as a block in the analysis. Fisher's-protected least significant difference (LSD) test was used to determine differences between treatments.

3. Results

3.1 Feeding period (3 to 6 weeks of age)

Average daily gain (ADG) and FI were not affected by treatment (Table 2). However, pigs fed the YPC diet had a lower FCR compared to those fed the CON, INS or NCL diets (P=0.028). There was no difference in the FCR between pigs fed the GLU diet or the YPC diet.

3.2 Lifetime performance

Apart from those pigs that were euthanased 21 days after weaning, the other pigs remained on the experiment for 118 days. The initial weight and final weight were not different between diets (Table 3). ADG, FI and FCR were not different between day 21 to day 118. However, there was a trend for pigs receiving the NCL diet to have a lower FCR between day 21 to day 118 compared to pigs fed the other diets (P=0.072). There was no difference in carcass weight, dressing percentage or P2 backfat depth among experimental diets (Table 3).

3.3 Small intestinal histology

Villous height in the duodenum varied between the treatments with pigs fed the YPC treatment having higher villi (P=0.029) compared to the CON and INS treatments (Table 4). There was no difference in villous height between pigs fed the GLU and NCL diet compared to the YPC diet. There was also no difference in any other small intestinal index examined.

3.4 Haptoglobin and immunoglobulin concentrations

Haptoglobin concentration at 7 and 21 days after weaning was not affected by treatment (Table 5). Haptoglobin concentration decreased from day 7 to day 21. IgA and IgM were not different between any of the experimental diets on either day 7 or day 21 after weaning. While there was no difference between diets for IgG on day 7, by day 21 the IgG levels were increased in pigs that received the INS and GLU diets compared to the CON and NCL diets (P=0.034). There was no difference in the IgG level in the YPC diet compared to the other diets. From day 7 to day 21 IgA and IgM levels were increased while IgG was decreased.

4. Discussion

Pig performance was generally unaffected by diet, although pigs fed the YPC diet had a lower FCR than other treatments in the weaner phase. There was also no difference in overall lifetime performance between the treatments. However, in a large scale commercial study (Barbara Frey, Consistent Pork, East Perth, unpublished) there was a long term benefit to growth performance which was not apparent in this experiment. Carlson et al. (2005) also found that pigs that received the YPC diet were 7 kg heavier than the CON diet at 130 days of age.

The lack of any statistically significant difference in growth performance between treatments is unlikely to have been due to insufficient replication as there were a relatively large number of animals per treatment. It is more likely that the conditions under which these pigs were reared were close to optimal, during both the

period after weaning and during the grower-finisher phase. While there would have been some stressors related to the practice of weaning, the extent to which this affected subsequent performance would have been less then would be experienced in typical commercial piggeries. This concurs with Mateo (2005) who found no effect of nucleotide supplementation on pig performance and proposed that is was likely that the pigs in the experiment were not as challenged by disease and were less stressed than commercial pigs.

Pigs that received the YPC, GLU and NCL diet had an increased villous height in the duodenum. This finding is in agreement with Mateo (2005) and Martinez-Puig et al. (2007) who, when feeding nucleotide supplemented diets, found an increase in villous height. Ewtushik et al. (2000) also found an increase in villous height in the duodenum when pigs were fed GLU. In contrast, Carlson et al. (2005) found no difference in the duodenal villous height but a reduced crypt depth in pigs that received a YPC diet. The increase in intestinal tissue growth could be due to the presence of nucleotides in the YPC and nucleotide diets as nucleotides are the building blocks of nucleic acids that are required for new tissue growth (Mateo, 2005). In addition, the increased villous height suggests that more cells were migrating to the villi to aid in digestion and absorption and this may assist the pig in overcoming the negative consequences of weaning (Carlson et al. 2005).

The lack of difference in daily gain and feed intake but a greater villous height in the nucleotide diet concurs with work by Martinez-Puig et al. (2007) and Kehoe et al. (2008), both of whom reported that nucleotide supplementation of the diet for pigs and calves, respectively, improved villous height with no impact on daily gain and

feed intake. Martinez-Puig et al. (2007) suggested that the incidence of post-weaning diarrhoea was reduced through supplementation with nucleotides although this was not observed in this experiment due to the low incidence of diarrhoea. This may have been due to the optimum conditions in which the pigs were housed.

Immunoglobulin G was increased in the serum of pigs that received INS and GLU diets. It was also thought that IgG levels might have been increased in pigs that received the nucleotide diet as nucleotides play an important role in an efficient immune system function (Rutz *et al.* 2006). However, Mateo (2005) also found no difference in serum IgG when the diet was supplemented with nucleotides and suggested that this may have been due to an absence of a challenge or that the nucleotides were not included in the diet for a sufficient period.

4. Conclusions

This experiment tried to elucidate which of the factors of the YPC diet was contributing to the beneficial response in growth performance and time to slaughter that has been found previously. Although no benefit was found in daily gain and feed intake in this experiment when inositol, glutamate and nucleotides were used either separately or together, positive responses were found in the feed conversion ratio, duodenal histology and the IgG concentration depending on the additive. The YPC diet resulted in an improvement in duodenal histology and FCR in the first 3 weeks post-weaning. While GLU and NCL supplementation numerically improved duodenal villous height, INS and GLU supplementation increased IgG levels at 21 days post-weaning. Although these results failed to show which components of YPN are

284 responsible for the positive effect of YPN in a weaner diet, the demonstrated positive
285 effect of YPN, INS, GLU and NCL may suggest that these components may have
286 specific effects on intestinal histology and immunity.
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Table 1: Composition of the experimental diets (g/kg, as-fed basis).

Phase	Weaner 1					Weaner 2				
Treatment[a]	CON	INS	GLU	NCL	YPC	CON	INS	GLU	NCL	YPC
Ingredients										
Wheat	433.3	433.2	430.6	431.3	422.9	586.0	586.0	584.4	584.8	557.0
Cooked oats	150.0	150.0	150.0	150.0	150.0	100.0	100.0	100.0	100.0	133.3
Full fat soya	100.0	100.0	100.0	100.0	100.0	96.4	96.4	96.4	96.4	73.3
SPC (650 g CP/kg)[b]	80.4	80.4	80.4	80.4	52.7	10.0	10.0	10.0	10.0	10.0
Fishmeal (600 g CP/kg)	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Whey powder	80.0	80.0	80.0	80.0	80.0	30.0	30.0	30.0	30.0	30.0
Meat meal (500 g CP/kg)	41.6	41.6	41.6	41.6	38.7	48.7	48.7	48.7	48.7	47.7
Vegetable oil	5.0	5.0	5.0	5.0	5.0	0	0	0	0	0
Vitamin-mineral[c]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
L-Lysine	1.046	1.046	1.046	1.046	1.138	1.293	1.293	1.293	1.293	1.177
DL-Methionine	0.055	0.055	0.055	0.055	0.022	0.043	0.043	0.043	0.043	0
L-Threonine	0.491	0.491	0.491	0.491	0.434	0.520	0.520	0.520	0.520	0.426
Limestone	1.00	1.00	1.00	1.00	2.04	0	0	0	0	0
Sodium chloride	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Enzyme complex[d]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Betaine[e]	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Organic acid[f]	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Inositol[g]	0	0.122	0	0	0	0	0.073	0	0	0
Glutamate[g]	0	0	2.7	0	0	0	0	1.6	0	0
Nucleotides[h]	0	0	0	2.0	0	0	0	0	1.2	0
YPC[i]	0	0	0	0	40.0	0	0	0	0	20.0
Calculated composition										
Digestible Energy (MJ/kg)	15.2	15.2	15.2	15.2	15.1	14.9	14.9	14.9	14.9	14.8
Crude protein	257	257	257	257	257	237	237	237	237	239
SID[j] Lysine	13.7	13.7	13.7	13.7	13.6	12.6	12.6	12.6	12.6	12.6
SID Met + Cys	7.6	7.6	7.6	7.6	7.5	7.0	7.0	7.0	7.0	6.9
SID Threonine	8.7	8.7	8.7	8.7	8.6	8.0	8.0	8.0	8.0	7.9
Available phosphorus	5.3	5.3	5.3	5.3	5.5	5.4	5.4	5.4	5.4	5.5
Calcium	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	9.9
Ash	72	72	72	72	70	59	59	59	59	58

[a]CON: control, INS: inositol, GLU: glutamate, NCL: nucleotides, YPC: Yeast protein concentrate.

[b]Soy protein concentrate (Soycomil, ADM Specialty Ingredients, Koong aan de Zaan, Netherlands).

[c]Provided the following nutrients (per kg of air-dry diet):Vitamins: A 7000 IU, D_3 1400 IU, E 20 mg, K 1 mg, B_1 1 mg, B_2 3 mg, B_6 1.5 mg, B_{12} 15 µg, Calcium pantothenate 10.7 mg, Folic acid 0.2 mg, Niacin 12 mg, Biotin 30 µg. Minerals: Co 0.2mg (as cobalt sulphate), Cu 10 mg(as copper sulphate), Iodine 0.5 mg (as potassium iodine), Iron 60 mg (as Ferrous sulphate), Mn 40 mg (as manganous oxide), Se 0.3 mg (as Sodium Selenite). Zn 100 mg (as zinc oxide) and 20 mg antioxidant as Endox (Kemin Industries Inc., Des Moines, USA).

[d]Rovabio®™ (Adisseo, Singapore). Active enzymes: xylanase, β-glucanase, cellulase, pectinases, protease.

[e]Betafin (Danisco, Marlborough, UK)

[f]Acid-Pak 4-way (Alltech Inc, Kentucky, USA). Provides sorbic and citric acids, *Lactobacillus acidophilus*, *Enterococcus faecium*, protease and cellulase.

[g]Sigma-Aldrich Pty Ltd, St Louis, Mo, USA.

[h]ASCOGEN, purified nucleotides from the *Saccharomyces cervisiae* (Chemoforma Ltd, Augst, Switzerland).

[i]Nupro (Alltech Inc., Kentucky, USA) is cell contents of the *Saccharomyces cervisiae* which contains 3.6 g inositol, 67.6 g glutamate and 70 g nucleotides per kg product. The 40 g and 20 g YPC in phase 1 and 2 diets, respectively, provides the same levels of inositol, glutamate and nucleotides supplemented in INS, GLU, and NCL diets, respectively.

[j]SID: standardised ileal digestible.

Table 2: Liveweight (LW), average daily gain (ADG), voluntary feed intake (FI) and feed conversion ratio (FCR) for weaner pigs fed experimental diets for the first three weeks post-weaning (3-6 weeks of age)[a].

Treatment[b]	CON	INS	GLU	NCL	YPC	SED[c]	P-value
n	18	18	18	18	18		
LW (kg):							
Day 0	6.14	6.13	6.13	6.14	6.14	0.097	1.00
Day 21	10.1	10.1	10.7	10.1	10.6	0.702	0.216
ADG (g)	190	187	217	189	214	15.5	0.150
FI (g/day)	363	360	393	367	348	19.3	0.221
FCR	1.97[a]	2.00[a]	1.84[ab]	2.04[a]	1.72[b]	0.108	0.028

[a]n=18.
[b]CON: control, INS: inositol, GLU: glutamate, NCL: nucleotides, YPC: Yeast protein concentrate.
[c]Standard error of the difference

Table 3: Live weight (LW), average daily gain (ADG), voluntary feed intake (FI), feed conversion ratio (FCR) and carcass quality for those pigs that were on the experiment from weaning until slaughter 118 days later[a].

Treatment[b]	CON	INS	GLU	NCL	YPC	SED[c]	p-value
Days on trial	118	118	118	118	118		
LW d-118 (kg)	101.5	98.6	100.9	100.9	101.5	2.47	0.752
ADG d-21 to d-118 (g)	943	908	927	937	934	23.4	0.637
FI d-21 to d-118 (kg/d)	2.29	2.19	2.28	2.17	2.27	0.060	0.137
FCR d-21 to d-118	2.43	2.41	2.46	2.31	2.44	0.053	0.072
Carcass weight (kg)	65.1	63.2	65.0	64.7	64.4	1.83	0.830
Dressing percentage	64.2	64.1	64.5	64.1	64.0	0.667	0.959
Backfat (P2, mm)	11.9	11.3	12.3	12.2	11.8	1.25	0.927

[a]n=10.
[b]CON: control, INS: inositol, GLU: glutamate, NCL: nucleotides, YPC: Yeast protein concentrate.
[b]Standard error of the difference

Table 4: Small intestine histology of pigs that received the experimental diets for 21 days after weaning[a].

Treatment[b]	CON	INS	GLU	NCL	YPC	SED[c]	P-value
Duodenum							
Villous height (μm)	465[a]	461[a]	517[ab]	521[ab]	569[b]	36.0	0.029
Crypt depth (μm)	211	233	259	225	231	24.4	0.422
VH:CD ratio	2.39	2.16	2.09	2.50	2.59	0.255	0.247
Jejenum							
Villous height (μm)	410	438	467	423	433	31.0	0.464
Crypt depth (μm)	236	241	227	224	231	14.3	0.752
VH:CD ratio	1.85	1.89	2.19	1.99	2.20	0.251	0.501
Ileum							
Villous height (μm)	375	389	405	399	410	26.0	0.674
Crypt depth (μm)	220	201	201	224	207	18.4	0.640
VH:CD ratio	1.85	2.13	2.13	1.93	2.22	0.249	0.552

[a]n=8.

[b]CON: control, INS: inositol, GLU: glutamate, NCL: nucleotides, YPC: Yeast protein concentrate.

[c]Standard error of the difference

413 Table 5: Haptoglobin and immunoglobulin concentrations in serum of pigs after
414 receiving the experimental diets for 7 and 21 days post-weaning[a].

Treatment[b]	CON	INS	GLU	NCL	YPC	SED[c]	P-value
Haptoglobin (mg/mL)							
Day 7	1.45	2.14	1.24	1.45	1.55	0.479	0.407
Day 21	0.759	1.06	0.514	0.574	0.864	0.230	0.142
Immunoglobulin A (mg/mL)							
Day 7	0.151	0.157	0.230	0.150	0.158	0.036	0.149
Day 21	0.465	0.384	0.387	0.338	0.399	0.087	0.698
Immunoglobulin G (mg/mL)							
Day 7	5.11	6.93	7.12	6.27	6.89	0.927	0.204
Day 21	3.57[a]	4.56[b]	4.37[b]	3.47[a]	4.05[ab]	0.395	0.034
Immunoglobulin M (mg/mL)							
Day 7	1.12	1.05	0.97	1.11	0.96	0.156	0.775
Day 21	1.89	2.12	1.73	1.67	1.91	0.242	0.376

415 [a]n=8.
416 [b]CON: control, INS: inositol, GLU: glutamate, NCL: nucleotides, YPC: Yeast protein
417 concentrate.
418 [c]Standard error of the difference
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