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Effects of an Enzymatically Hydrolyzed Yeast and Yeast Culture Combined with Flavomycin and Monensin on Finishing Broiler Chickens

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Abstract: An experiment was carried out to evaluate the relationship of an Enzymatically Hydrolyzed Yeast (EHY) and yeast culture combined with flavomycin and monensin (F + M) under two levels of Digestible Lysine (DLYS) on the growth performance, carcass measurements, nutrient retention and AMEn in broilers. One hundred and thirty two 35 d old Ross B308 male broilers individually housed in holding cages were used. Broilers were assigned to a factorial arrangement of 2 levels of EHY (0 and 1 kg/ton of feed) x 2 levels of F + M (0.0 and 0.05,0.50 kg/ton) x 2 DLYS levels (0.85 and 0.95%). The experiment lasted 2 wks. Results were subjected to analysis of varianza. EHY-fed broilers showed a trend for greater ($p<0.10$) feed intake, had improved ($p<0.05$) weight gain, feed conversion ratio and had greater ($p<0.01$) weight and yield of the carcass, breast and legs. Broilers fed EHY and 0.95% DLYS had higher nitrogen intake and retention and lower AMEn than those with 0.85% DLYS (EHY and DLYS interaction, $p<0.05$). EHY-fed broilers also had greater dry matter and ashes intake ($p<0.01$) and retention ($p<0.05$). The addition of F+M to the diet improved the weight gain ($p<0.05$), feed conversion ratio ($p<0.01$), nitrogen retention and AMEn ($p<0.05$). Broilers fed the 0.95% DLYS diet had greater feed conversion ($p<0.05$). The addition of EHY and F+M improved the production and nutrient use in broilers but their effects were independent. The EHY and DLYS showed an additive or synergistic relationship on the nitrogen intake and retention and AMEn.

Key words: Broiler, enzymatically hydrolyzed yeast and yeast culture, flavomycin plus monensin

INTRODUCTION

There is an abundance data on the scientific literature that has been presented on the benefits of antibiotics on the growth and health of poultry (Bedford and Fothergill, 2002; Ferket *et al*., 2002; Hooge *et al*., 2003). However, it has been argued that after long use, antibiotic growth promoters may lead to potential development of resistant pathogenic microorganisms which may represent a hazard for the consumer health (Phillips, 1999; Ratcliff, 2000). For this reason, it has been suggested that the poultry industry must develop alternatives to avoid using antibiotic growth promotants, or at least substantially reduce the amount of antibiotics used to maintain efficient poultry production and obtain safe poultry meat and egg products (Ferket *et al*., 2002). In Europe the banning of most of the antibiotic growth promoters have led to the outbreaks of different diseases caused by pathogenic microorganisms which may also represent a health risk for humans (Mateos and Lazaro, 2001; Williams, 2005; Cepero, 2005). Therefore, it is advisable to conduct research aimed to identify the conditions in which 1) antibiotic growth promoters are fully replaced or, 2) the effects of antibiotics are strengthening when strategically combined with non-antibiotic growth promoters. In several studies, additive or synergistic beneficial effects have been evaluated with some antibiotics that were used in combination with other non-antibiotic growth promoters such as plant extracts and essential oils (Hernandez *et al*., 2004; Lee *et al*., 2004; Barreto *et al*., 2008), organic acids (Chowdhury *et al*., 2009; Samanta *et al*., 2010), direct fed microbials (Hooge *et al*., 2004; Opalinski *et al*., 2007; Barreto *et al*., 2008) and MOS (Waldroup *et al*., 2003; Hooge *et al*., 2003; Hooge, 2004; Rosen, 2007) on broiler performance.

MOS is the additive most extensively studied as candidate to replace antibiotics. In a Meta-analysis that included 21 pen trials it was concluded that broilers fed MOS-diets had comparable body weight and feed conversion but lower mortality than those fed antibiotic-supplemented diets (Hooge, 2004). More recently, in a Holo-analysis that included 32 publications on the use of MOS as broiler feed additive it was confirmed the beneficial effects of MOS on broiler performance (Rosen, 2007). Since there are several sources of MOS and other yeast derivatives from Saccharomyces cerevisiae (Sc)

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available for broilers, it is important to test the effectiveness of these products against or in combination with antibiotic growth promoters.

It is proposed that the benefits of MOS is due to the presence of mannose units that binds potential pathogenic bacteria, particularly those with type 1 fimbria on their surface, blocking their adhesion and colonization of the intestinal cells (Mul and Perry, 1994). Also, MOS favors the growth of beneficial bacteria such as *Lactobacillus* which are capable of neutralizing some enterotoxins and inhibiting the growth of pathogenic bacteria like *E. coli*, *Clostridium* and *Streptococcus* by producing organic acids and reducing the intestinal pH (Spring *et al*., 2000). This can lead to better gut health and increased nutrient availability resulting in enhanced growth and health as seen by yeast products (Radecki and Yokoyama, 1991; Stanley *et al*., 2004).

There is also considerable evidence showing that whole cells and several derivatives from Sc such as Yeast Culture (YC), Yeast Extracts (YE) and yeast fermentation products, that retain the cell wall components, exert similar beneficial effects as MOS on the growth and feed conversion ratio of poultry (Stanley *et al*., 2004; Zhang *et al*., 2005; Gao *et al*., 2009). A YC contains, in addition to yeast cells, metabolites such as peptides, organic acids, oligosaccharides, amino acids, flavor and aromatic substances and possibly some unidentified growth factors, which have been proposed to produce beneficial responses in poultry production (Gao *et al*., 2009; Zhang *et al*., 2005). A YE is also derived from the cell content of live yeast and contain high levels of nucleotides, inositol and glutamic acid and have also resulted in beneficial effects on the feed conversion of broilers (Rutz *et al*., 2006; Silva *et al*., 2009).

Celmanax® is a trade mark of Vi-Cor® (Mason City, IA, USA) that contains Enzymatically Hydrolyzed Yeast (EHY), YE and YC with the cell wall components and the metabolites of the cell content recommended for all classes of livestock. Celmanax® also contains yeast mannans and provides the metabolites normally found in YC and can replace yeast-based products in the diet. The main issue addressed in the present study was to elucidate if there was any additive or synergistic relationship between the EHY and YC residues present in Celmanax® and F + M, which are the antibiotic growth promoter and the anticoccidial drug, respectively, normally used in diets for poultry. The other issue considered in this study was to clarify whether there was an increased need of dietary amino acids in broilers that have an enhanced growth rate due to the addition of antibiotic or non-antibiotic growth promoters or their combination in the diet. Therefore, an experiment was carried out to evaluate the relationship of an Enzymatically Hydrolyzed Yeast (EHY) and yeast culture combined with Flavomycin and Monensin (F + M) under two levels of Digestible Lysine (DLYS) on the growth performance, carcass measurements, nutrient retention and AMEn in broilers.

Table 1: Composition of experimental diets

	Digestible lysine (%)			
Items	0.85		0.95	
Sorghum	64.27		62.27	
Soybean meal	27.00		26.50	
Canola meal	0.00		0.00	
Corn gluten meal	0.00		0.00	
Vegetable oil	4.44		4.44	
Calcium phosphate	1.72		1.72	
Calcium carbonate	1.16		1.16	
Sodium bicarbonate	0.31		0.31	
Salt, - NaCl - I	0.30		0.30	
L-Lysine-HCL	0.00		1.30	
L-Threonine	0.00		0.70	
DL-Methionine	0.10		0.50	
Vitamins and minerals[1]	0.70		0.70	
Calculated analysis				
ME (kcal/kg)	3.20		3.20	
PC (%)	17.30		17.30	
Ca (%)	0.85		0.85	
Avail. P (%)	0.42		0.42	
Digestible amino acids	%	AAP	%	AAP
Lysine	0.85	-	0.95	-
Threonine	0.57	0.67	0.64	0.67
Methionine	0.35	0.41	0.39	0.41
Tryptophane	0.20	0.23	0.20	0.21
Arginine	1.07	1.26	1.07	1.13
Valine	0.78	0.92	0.78	0.82
Isoleucine	0.70	0.82	0.70	0.74

[1]Each kg provides: 6 500 IU of vitamin A; 2 000 IPU of vitamin D_3; 15 IU of vitamin E; 1.5 mg of vitamin K; 1.5 mg of thiamine; 5 mg of riboflavin; 35 mg of niacin; 3.5 mg of pyridoxine; 10 mg of pantothenic acid; 1500 mg of choline; 0.6 mg of folic acid; 0.15 mg of biotine; 0.15 mg of vitamin B_{12}; 100 mg of Mn; 100 mg of Zn; 50 mg of Fe; 10 mg of Cu; 1 mg of I. AAP = AA Profile

MATERIALS AND METHODS

The research was carried out in the facilities of the Experimental Poultry Unit of the National Center of Disciplinary Research in Animal Physiology-INIFAP, located in Querétaro, México. One hundred and thirty two 35 d old Ross B308 male broilers individually housed in holding mesh-floored cages with a metal feeder and a cup drinker in a naturally ventilated unit was used. Broilers were assigned to eight treatments in a complete randomized design with a factorial arrangement of 2 levels of EHY (0 and 1 kg/ton of feed) x 2 levels of F + M (0.0 and 0.05,0.50 kg/ton) x 2 DLYS levels (0.85 and 0.95%). The F + M doses used were equivalent to 4 and 100 ppm, respectively. Diet 1 was formulated with 0.85% DLYS (Table 1) and the content of the rest amino acids was adjusted according to the ideal profile suggested by Baker and Chung (1992). Diet 2 was similar to diet 1, plus the addition of crystalline lysine to reach a level of 0.95% DLYS. Crystalline methionine and threonine were also included to keep the same profile of these amino acids as Diet 1.

Feed and water were offered *ad libitum* for the two weeks of the study. During the last three days, all excreta was collected every 24-h using clean stainless steel collection trays, weighed and stored frozen at -20°C in polyethylene bags. The last day, all broilers were killed by cervical dislocation. The abdominal cavity was opened to excise the liver, small and large intestine and cecum. Organs were rinsed with clean water, dried with paper towels and weighed. The carcass was dissected and cut in its main parts (breast, legs, thighs and ribs) then dried and weighed.

Excreta samples were lyophilized and ground using a 2 mm mesh. Determinations of dry matter, ashes, nitrogen and energy were done in excreta and diets following standard procedures (AOAC, 2002). Then, the retention of nutrients was estimated.

Results were subjected to ANOVA by using the GLM procedures (SAS, 1999). For all response variables the experimental unit was each bird. Percentage results were transformed to arcsine values before analysis.

RESULTS AND DISCUSSION

Growth performance and carcass measurements: No additive or synergistic relationship was detected between EHY and F + M, EHY and DLYS, F + M and DLYS and EHY, F + M and DLYS in any of the growth and carcass measurements as any of the interaction tested was not statistically significant.

EHY-fed broilers showed a trend ($p<0.10$) for greater feed intake and had improved ($p<0.05$) weight gain and feed conversion ratio (Table 2), which is in agreement with previous results observed in broiler fed diets supplemented with yeast. In a meta-analysis of trials of MOS-fed broilers a positive effect of MOS was observed in 80% of the studies, with an average improvement of 1.6% and 1.99% on weight gain and on feed conversion ratio, respectively (Hooge, 2004). Feed intake, weight gain and feed conversion were improved by 3.3, 6.9 and 3.8% in EHY-fed broilers, respectively. Improved growth performance have been reported in broilers fed other yeast products such as YC, YC residue, YE, whole cells, cell wall components or a fermentation product from Sc (Gao *et al*., 2008; 2009; Stanley *et al*., 2004; Zhang *et al*., 2005).

EHY-fed broiler also showed greater ($p<0.05$) weight and yield of the thighs and had greater ($p<0.01$) weight and yield of the carcass, breast and legs (Table 2) which is in agreement to some results observed in broiler fed yeast added diets. Clementino Dos Santos *et al*. (2002) reported increased breast yield in MOS-fed broilers. The addition of dried yeast in broilers resulted in higher carcass weight and yield (Adejuno *et al*., 1999; Miazzo *et al*., 2005). Also, the inclusion of live yeast increased the carcass yield in free-range chickens (Pelicia *et al*., 2004).

The addition of F + M to the diet improved the weight gain ($p<0.05$) and feed conversion ratio ($p<0.01$) by 5.2% and 6.7%, respectively (Table 2). It has been reported that antibiotic growth promoters improve the growth of broiler between 5 and 10% and the feed conversion between 2 and 3% (Walton, 1996; Hooge, 2004). In studies in which flavomycin was combined with nicarbazin and monensin or monensin alone a 3.7 and 5.0% improvement in body weight and feed conversion ratio was observed (Roch, 1999; Perez *et al*., 2002).

Broilers fed the 0.95% DLYS diet showed better feed conversion ratio ($p<0.05$) and had an arithmetic improvement on the weight gain (Table 2). In other reports it has been observed that increasing lysine intake above the level required for maximum live weight gain affects body composition by increasing breast meat yield and decreasing abdominal fat percentage which can lead to beneficial effects on feed conversion ratio (Grisoni, 1991; Leclercq, 1998; Rezaei *et al*., 2004).

Table 2: Productive parameters, weight and yield of carcass and its components in broilers fed a yeast culture and enzymatically hydrolyzed yeast, with or without flavomycin and monensin and two levels of digestible lysine

	EHY (kg/ton)			F + M (kg/ton)			DLYS (%)		
	0.0	1.0	SEM[a]	0.0	0.05,0.50	SEM[a]	0.85	0.95	SEM[a]
Productive parameters									
Feed intake (g/d)	180.0^{b}	185.9^{c}	2.508	183.1	182.8	2.43	183.2	182.6	2.539
Weight gain (g/d)	76.3^{d}	81.6^{e}	1.576	77.0^{h}	81.0^{i}	2.59	77.3	80.6	1.595
Feed efficiency	2.41^{b}	2.29^{c}	0.040	2.44^{j}	2.27^{k}	0.041	2.42^{l}	2.29^{m}	0.042
Weight of the carcass and its components (g)									
Carcass	1099.1^{f}	1190.2^{g}	23.987	1151.0	1138.4	23.472	1143.1	1146.3	24.367
Breast	450.9^{j}	497.7^{g}	11.532	479.9	468.7	11.284	472.8	475.8	11.715
Thighs	328.7^{d}	349.9^{e}	7.445	339.8	338.9	7.285	341.1	337.5	7.563
Legs	204.7^{f}	220.6^{g}	4.124	210.4	214.9	4.035	214.9	210.4	4.189
Ribs	114.6	121.9	4.129	120.7	115.8	4.040	114.1	122.4	4.194
Relative yield of carcass and its components (%)									
Carcass	42.9^{f}	46.4^{g}	0.937	44.9	44.4	0.916	44.6	44.7	0.951
Breast	17.6^{f}	19.4^{kg}	0.450	18.7	18.3	0.440	18.4	18.5	0.457
Thighs	12.8^{d}	13.6^{e}	0.291	13.2	13.2	0.284	13.3	13.1	0.295
Legs	8.0^{f}	8.6^{g}	0.161	8.2	8.3	0.157	8.3	8.2	0.163
Ribs	4.4	4.7	0.1614	4.7	4.5	0.158	4.4	4.7	0.163

[a]Standard error of the mean. [b-c]Effect of EYH, $p<0.10$. [d-e]Effect of EYH, $p<0.05$. [f-g]Effect of EYH, $p<0.01$. [h-i]Effect of F+M, $p<0.10$. [j-k]Effect of F+M, $p<0.01$. [l-m]Effect of DLYS, $p<0.05$

Table 3: Nutrient balance and weight of digestive organs in broilers fed a yeast culture and enzymatically hydrolyzed yeast, with or without flavomycin and monensin and two levels of digestible lysine

	EHY (kg/ton)			F + M (kg/ton)			DLYS (%)		
	0.0	1.0	SEM[a]	0	0.05,0.50	SEM[a]	0.85	0.95	SEM[a]
Dry matter									
Intake (g/d)	187.2^{b}	206.5^{c}	4.283	196.1	197.6	4.374	196.3	197.3	4.351
Excretion (g/d)	20.4	20.6	0.476	20.2	20.9	0.472	20.9	20.2	0.490
Retention (%)	88.9^{d}	89.8^{e}	0.353	89.7	89.0	0.358	89.3	89.4	0.363
Ashes									
Intake (g/d)	12.4^{b}	13.7^{c}	0.284	13.0	13.1	0.290	13.0	13.1	0.289
Excretion (g/d)	3.0	3.0	0.073	2.9^{f}	3.2^{g}	0.073	3.0	3.0	0.075
Retention (%)	75.0^{d}	77.2^{e}	0.893	77.4^{f}	74.8^{g}	0.907	76.3	75.9	0.920
Nitrogen									
Intake (g/d[h])	4.2	4.4	0.069	4.16^{f}	4.41^{g}	0.069	4.3	4.3	0.069
Excretion (g/d)	0.9	0.9	0.028	1.0	0.9	0.028	1.0^{k}	0.9^{l}	0.027
Retention (%[h])	77.6	79.0	0.733	77.0^{f}	79.7^{g}	0.734	77.1	79.6	0.734
Energy									
Intake (g/d)	635.3^{d}	667.3^{e}	10.509	661.2	641.4	10.503	667.0^{k}	635.6^{l}	10.508
Excretion (g/d)	78.6	79.6	1.623	78.3	79.8	1.613	82.3^{k}	75.8^{l}	1.662
AMEn[h]	3438.0	3450.0	9.764	3429.0^{f}	3458.0^{g}	9.762	3453.0	3434.0	9.758

[a]Standard error of the mean. $^{b-c}$Effect of EHY, p<0.01. $^{d-e}$Effect of EHY, p<0.05. $^{f-g}$Effect of F+M, p<0.05. [h]Interaction EHY and DLYS, p<0.05. $^{k-l}$Effect of DLYS, p<0.05

However, in this study there was not any statistical difference on the weight and yield of the carcass and its components between DLYS levels.

The addition of EHY combined with F + M did not affect any of the response variables suggesting that their effects are independent. In very few studies additive or synergistic effects of MOS and antibiotics have been shown (Mathis, 2000; Sefton *et al*., 2002; Hooge *et al*., 2003). Even though the growth of EHY- and F + M-fed broilers was improved, the addition of supplemental lysine to the diet did not result in additional benefits on growth rate nor on carcass yield, suggesting that the levels of amino acids supplied in the diet with 0.85% of DLYS was enough to sustain an adequate growth of the broilers.

Nutrient balance: A two way interaction (EHY and DLYS, p<0.05) on the nitrogen intake and retention and the AMEn was observed (Table 3). Regardless of the DLYS level, broilers fed the diet without EHY had similar nitrogen intake and retention and AMEn (Fig. 1), but broilers fed EHY and 0.95% DLYS had higher nitrogen intake and retention and lower AMEn than those with 0.85% DLYS. In previous research it was shown that lower nitrogen intake in broilers caused similar or better nitrogen retention compared to broilers that had higher nitrogen intake (Kerr and Kidd, 1999; Adeola and Sands, 2004). Opposite to this, in the present research, EHY elicited both, greater nitrogen intake and retention at a higher dietary lysine (0.95% DLYS).

EHY-fed broilers, regardless of the dietary lysine, had higher energy intake (p<0.05) but only those fed the 0.85% DLYS diet had higher AMEn. It is probably that broilers fed the 0.95% DLYS diet with EHY had greater nitrogen turnover due to their greater nitrogen intake and retention which may have caused greater energy expenditure in such a way that the benefits of higher nitrogen retention may have been counterbalanced by the increased metabolic rate elicited by the extra nitrogen in the body.

EHY-fed broiler also showed greater intake (p<0.01) and retention (p<0.05) of dry matter and ashes (Table 3). These results agree with previous reports in turkey and broilers. In turkey poults fed YC improved GE and mineral retention was reported (Bradley and Savage, 1995). In male turkeys the inclusion of MOS increased the AME by 2.94% (Ferket *et al*., 2002). In MOS-fed broiler, a trend for improvement AME and NE was observed compared to broilers fed a control diet (Yang *et al*., 2008). Also, broilers fed diets supplemented with dried yeast showed higher apparent retention of dry matter, crude protein, ether extract, crude fibre, hemicellulose, cellulose and neutral detergent fibre than broilers fed a control diet (Onifade and Babatunde, 1996). However, in other studies no differences were reported on the use of energy and other nutrients in MOS-fed broilers (Hughes, 2003; Yang *et al*., 2007).

In spite that there was an additive or synergistic relationship between EHY and DLYS on the nitrogen retention and AMEn, this was not reflected in any of the productive responses. The explanation for this is unclear. It remains to evaluate whether increasing the experimental days or by rearing broilers in floor-pens these effects are strengthen.

With the addition of F + M (Table 3) the ashes retention decreased but the nitrogen intake and retention and the AMEn increased (p<0.05). These findings agree with previous reports in which improvements on carbohydrate and fat digestion and nitrogen and energy retention in broilers fed antibiotic growth promoters were observed (Buresh *et al*., 1985; Harms *et al*., 1986; Anderson *et al*., 1999).

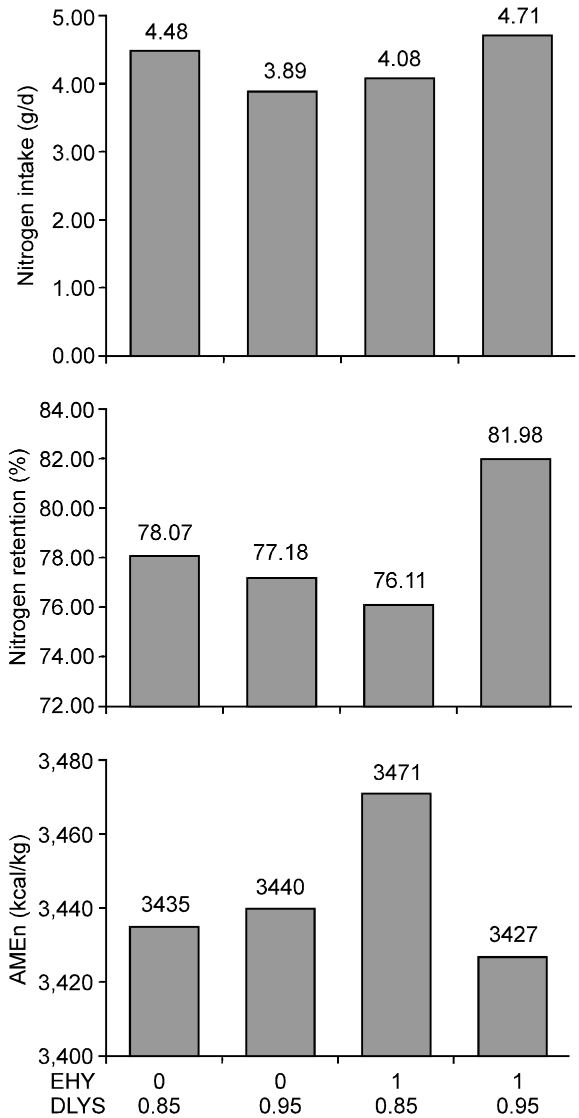

Fig. 1: Relationship between EHY and DLYS on the nitrogen intake, nitrogen retention and AMEn

Weight of the organs: The weight of the organs was similar among treatments (Data not shown). In previous research no effects of MOS on the organ weight such as proventriculus/gizzard, small intestine, liver and pancreas have been observed (Iji *et al*., 2001; Bozkurt *et al*., 2008; 2009). With the use of antibiotic growth promoters, reductions on the weight and length of the intestine of broilers have been reported (Anderson *et al*., 1999; Postma *et al*., 1999) as consequence of the thinning of the digestive epithelium. This thinning of the epithelium is thought to be caused by the reduction of the enterocite turnover rate as result of a lower production of bacterial toxins (Bedford, 2000). The disagreements in regards to the results of the present study, in which no differences were seen on the weight of the organs are likely attributable to the fact that F + M were supplied for 14 days and that broilers were housed individually in holding cages which reduced their exposure to bacterial challenges.

The inclusion of EHY in the feeds of broilers improved feed intake, weight gain, feed conversion ratio, carcass and breast weight and yield, retention of nutrients and AMEn and also, the inclusion of F + M enhanced the weight gain, feed efficiency, nitrogen retention and AMEn. Even though the effects of EHY and F + M on the growth, nutrient retention and energy use by broilers were quite similar, no additive or synergistic relationship was observed between them. The addition of supplemental dietary lysine did not cause additional benefits on growth rate nor on carcass yield which suggest that a level of 0.85% of DLYS was enough to sustain an adequate growth of finisher broilers. In spite that there was an additive or synergistic relationship between EHY and DLYS on the nitrogen retention and AMEn, this was not reflected in any of the productive responses.

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