Growth Associated Hormones Response and Fat Metabolism Change in Finishing Pigs Fed with n-Methyl-d, L-Aspartate

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ABSTRACT : A trial was conducted to investigate the effect of dietary NMA on several growth associated hormones and fat metabolism in finishing pigs. A total of 84 crossbred finishing pigs (average initial BW of 56±0.37 kg) were divided into 6 pens, 14 pigs per pen (7 gilts and 7 barrows per pen). 3 pens of pigs were fed with control diet (corn-soybean meal) and the others were fed control diet addition with 50 mg/kg NMA. During the trial, all pigs were given free access to feed and water. After 44 days trial, 8 pigs from each treatment (4 gilts and 4 barrows, weight similar to average group weight, 86.94±0.71 kg for control group, and 90.55±1.51 kg for NMA treated group) were sacrificed to collect the sample of the liver, longissimus muscle, subcutaneous fat (10th rib). The addition of NMA in diet increased the IGF-I, Insulin, T3, T4 levels in serum by 50.68% ($p<0.05$), 38.36% ($p<0.05$), 123.33% ($p<0.01$), 60.58% ($p<0.03$), respectively. Meanwhile, IGF-I level in the liver and the muscle were increased with 17.83% ($p<0.03$) and 26.00% ($p<0.03$) with addition of NMA. The data from subcutaneous fat (10th rib) analysis showed that supplement of 50 mg/kg NMA decreased the total activities of malic dehydrogenase (MDH) by 20.54% ($p<0.05$), glucose-6- phosphate dehydrogenase (G-6-DPH) by 16.97% ($p<0.05$), and decreased the specific activities of MDH and G-6-DPH by 37.46% ($p<0.01$) and 35.06% ($p<0.01$), respectively. The hormone sensitive lipase (HSL) total activity was increased by 25.00% ($p<0.05$) in NMA treated pigs. These results indicated that addition of 50 mg/kg NMA to diet can induce the endocrine great change in finishing pigs, furthermore, inhibit the fat synthesis through suppressing lipogenic enzymes and promote the fat degradation by elevating HSL activity in finishing pigs. ***(Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 7 : 1026-1030)***

Key Words : NMA, Growth Associated Hormone, Fat Metabolism, Finishing Pigs

INTRODUCTION

Excitatory amino acids (EAA), such as glutamate and asparate, are major neurotransmitter in the mammalian central neurons system, which generally stimulate (primary via N-methyl-d-aspartate, NMDA receptor) the release of neuropeptides from the central nervous system, thereby influencing pituitary functions. Abundant studies have indicated that n-methyl-d,l-aspartate (NMA), an agonist of glutamate, increases pituitary secretion of LH and GH under appropriate conditions in a variety of domestic animal species (Estienne et al., 1996a, 1997). For example, NMA at an i.v. dose of 10 mg/kg BW increased circulating concentration of LH in prepubertal gilts, but not at dose of 1.25, 2.50 or 5.00 mg/kg BW (Estienne et al., 1995). More recently, Estienne et al. (1998) reported that NMA at a dose of 10 mg/kg BW, increased LH release in gilts during the luteal phase of the estrons cycle, but not during the follicular phase of the estrons cycle. In addition, vast researches have proved that NMA stimulates the pituitary secretion of GH *in vitro* and *in vivo* (Barb et al., 1993, 1996; Estienne et al., 1996b, 1998, 2000a).

Up to now, most of researches focus on the effect of NMA on LH and GH, particularly on reproduction associated hormone. There is little information about the effect of NMA on growth associated hormone except GH. Based on previous research reports, NMA stimulation of the secretion of GH is more consistent. Our hypothesis herein is that NMA would induce the growth associated hormone response other than LH and GH in pigs via hypothalamus-pituitary axis, and change the fat metabolism due to hormone status alteration. As our knowledge, there is no report investigating the growth associated hormone response and fat metabolism change in finishing pigs treated with dietary NMA.

MATERIALS AND METHODS

Animals and samples collection

Eighty four crossbred (Landrace×Yorkshire×Duroc) pigs, average initial BW of 56±0.37 kg (mean±SE), were divided into 6 pens, 14 pigs per pen (7 gilts and 7 barrows). Pigs were allowed *ad libitum* access to water and a corn-soy bean meal diet. This diet met or exceeded NRC (1998) recommendations for nutrients and was analyzed to provide 16.95% CP, 3.40% crude fat, 0.97% calcium, 0.52% total phosphorus, and was calculated to provide digestible energy concentration of 3,240 kcal/kg. Three pens pigs were fed control diet and the other 3 pens pigs were fed control diet addition with 50 mg/kg NMA (Based on our preliminary experiment, using growth performances as criterion,

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unpublished data). After 44 days trial, 8 pigs from each treatment (4 gilts and 4 barrows, weight similar to average group weight, 86.94±0.71 kg for control group, 90.55±1.51 kg for NMA treated group) were sacrificed to collect the sample of the liver, longissimus and subcutaneous fat (10th rib). Before the pigs were slaughtered, blood was sampled into glass tubes and allowed to clot overnight at 4°C. Serum was then harvested after centrifugation and stored at -20°C until assayed.

Radioimmunoassays

Serum concentration of insulin, free thyroxine (FT4) and free triiodothyronine (FT3) were measured with the RIA kits (Beijing North Immunological Institute, China) in a Gamma-counter (Packard 8500, USA). Intra- and inter-assay CV for insulin, FT3 and FT4 were 8.7% and 10.5%, 5.2% and 9.8%, 6.7% and 10.1%, respectively. Serum concentration of somatostatin (SS) was measured with the RIA kit (Provided by No 4 Military Medical University, China). Intra- and inter-assay CV for SS were 6.5% and 8.7%, respectively.

IGF-I was firstly extracted from the muscle and the liver as previous described procedure (D'Ercole and Underwood, 1987). Briefly, a 0.2 g portion of the tissue was homogenized in 3 ml of 4% acetic acid and centrifuged at 1,500 rpm for 10 min, 0.5 ml supernatant was taken and added 60 μl NaOH (5 mol/l) to adjust pH from 6 to 8. Then, RIA kit (Incstar Co. Ltd, USA) was used to measure the concentration of IGF-I in the muscle, the liver and the serum. Intra- and inter-assay for IGF-I in the serum, the muscle and the liver were 5.8% and 10.0%, 5.3% and 9.8%, 4.7% and 8.5%, respectively.

Enzymological analysis

The total and specific activities of malic dehydrogenase (MDH), glucose-6-phosphate dehydrogenase (G-6-PDH) as well as hormone sensitive lipase (HSL) in subcutaneous fat (10th rib) were determined spectrophotometrically as the previous described methods (Xi et al., 2001).

Statistical analyses

For each hormone concentration and individual enzyme activity, each slaughtered pig was used as an experimental unit. Data were analyzed by analysis of variance using the general linear model procedures of SAS (SAS, 1989). For all data, the model included treatment NMA as main effect. Comparison were considered significantly different if $p<0.05$.

RESULTS

The addition of NMA in diet significantly changed the status of growth associated hormone in finishing pigs. In NMA fed pigs, serum FT4 and FT3 concentrations were increased by 60.58% ($p<0.03$) and 123.33% ($p<0.01$) (table 1). Serum insulin concentration was increased from 7.56 μIU/ml to 10.46 μIU/ml (figure 1), with addition of NMA in the diet. Serum SS concentration in NMA fed pigs was increased by 7.05% ($p=0.65$), but no significant difference was observed. Table 2 showed that feeding NMA improved IGF-I concentration in the serum, the liver and the muscle by 50.68% ($p<0.05$), 17.83% ($p<0.03$) and 26.00% ($p<0.03$), respectively, compared to control group pigs.

Fat metabolic enzyme activities in subcutaneous adipose tissue were greatly changed when pigs were fed the diet with 50 mg/kg NMA. Table 3 showed that the total and specific activity of MDH, G-6PDH were decreased by 20.54% ($p<0.05$) and 37.46% ($p<0.01$), 16.97% ($p<0.05$) and 35.06% ($p<0.01$), respectively, with addition of NMA in the diet. The change of HSL activity was also detected in subcutaneous adipose tissue. The total activity of HSL was increased by 25.00% ($p<0.05$), and no significant difference was found in specific activity of HSL, compared NMA fed

Table 1. Effects of dietary NMA on concentration of FT3, FT4 and SS in serum of finishing pigs

	NMA (mg/kg)		SE
	0	50	
Free Triiodothyronine (ng/ml)	0.90[b]	2.01[a]	0.10
Free Thyroxine (ng/ml)	41.88[b]	67.25[a]	9.57
Somatostatin (pg/ml)	73.86	79.07	11.27

[a,b] Means with different superscripts within rows differ ($p<0.05$).

Table 2. Effects of dietary NMA on IGF-I concentration in the serum, liver and muscle of finishing pigs

	NMA (mg/kg)		SE
	0	50	
Serum (mmol/l)	11.60[b]	17.47[a]	1.07
Liver (mmol/l)	23.56[d]	27.82[c]	0.78
Muscle (mmol/l)	22.31[d]	28.11[c]	1.62

[a,b] Means with different superscripts within rows differ ($p<0.05$).
[c,d] Means with different superscripts within rows differ ($p<0.03$).

Table 3. Effects of dietary NMA on activity of MDH and G-6-PDH in subcutaneous fat of finishing pigs

	NMA (mg/kg)		SE
	0	50	
MDH			
Total activity (U/g)	106.04[b]	84.62[a]	7.67
Specific activity (U/mg)	10.41[d]	6.51[c]	0.92
G-6-PDH			
Total activity (U/g)	93.56[b]	77.68[a]	3.06
Specific activity (U/mg)	11.41[d]	7.41[c]	0.70

[a,b] Means with different superscripts within rows differ ($p<0.05$).
[c,d] Means with different superscripts within rows differ ($p<0.01$).

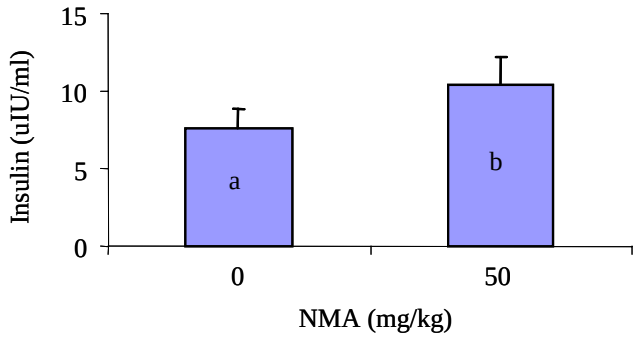

Figure 1. Effect of dietary NMA on concentration of insulin in serum of finishing pigs.

pigs and control group pigs (table 4).

DISCUSSION

In most of researches, animals were treated NMA with intravenously injection. Effects of NMA with i.v. injection on animal endocrine system, particularly in reproductive hormones, have been well documented (Estienne et al., 1997, 1999). Only few papers reported the effects of NMA on animals with oral administration. For example, in 1994, Harter-Dennis et al. conducted a trial in which NMA was fed to female broilers at doses of 0,125, 250, 375 and 500 mg/kg. They reported that feed conversion efficiency was improved by 5.4% in broilers fed NMA at a dose of 375 mg/kg, and the percentage of abdominal fat pad (ABF) and shell fat in carcasses was decreased a maximum of approximately 12.5% in broilers fed NMA at a level of 250 mg/kg. Most recently, Estienne et al. (2000b) reported a trial in which NMA was fed to barrows at level of 0, 100, 200, 300 mg/kg. However, they found that barrows fed NMA displayed poorer feed conversion efficiency and increased adipose tissue deposition. Currently, no experiment results could be used to postulate a possible mechanism for dietary NMA.

In addition, most of previous researches investigated the effects of NMA on LH and GH status in male and female pigs. The information about other hormones, particularly in growth associated hormone is quietly deficient. Therefore, the present study focused on the effect of dietary NMA on several important growth associated hormones status in finishing pigs, and, furthermore, investigated the change of several fat metabolic enzymes activities.

Table 4. Effects of dietary NMA on activity of HSL in subcutaneous fat of finishing pigs

	NMA (mg/kg)		SE
	0	50	
Total activity (U/g)	32.59[b]	40.56[a]	1.71
Specific activity (U/mg)	9.12	10.52	0.84

[a,b] Means with different superscripts within rows differ ($p<0.05$).

The results indicated the serum free thyroxine and triiodothyronine concentrations were significantly increased in pigs fed NMA with 50 mg/kg. This increase may be caused by the excitation of NMA on hypothalamus-pituitary axis. NMA may stimulate the secretion of thyroid-stimulating hormone (TSH) in hypothalamus, consequently, increase the secretion of thyotropin-releasing hormone (TRH). As a result, the circulating of T4 and T3 levels could be elevated with the stimulation of TRH. In addition, T3 concentration was proved to be elevated by GH treatment in pigs (Kirkwood et al., 1989). In present study, the serum insulin concentration was also improved by 38.36% ($p<0.05$) in finishing pigs fed NMA at a dose of 50 mg/kg. We hypothesize that this change may be attributed to the possible high level of GH in NMA treated pigs. Although we have no data about the GH level in NMA fed pigs, many previous researches reported that intravenous treatment of pigs with NMA significantly increased GH secretion *in vitro* and *in vivo* (Barb et al., 1993, 1996; Estienne et al., 1996b, 1998, 2000a). Moreover, strong evidences suggested that circulating insulin level could be elevated by growth hormone releasing factor and GH treatment in pigs (Walton et al., 1987; Gopinath et al., 1989; kirkwood et al., 1989; Johnson et al., 1990; Dubreuil et al., 1991; Klindt et al., 1995). Insulin is an anabolic hormone with potent effects on muscle protein metabolism. GH would decrease the insulin sensitivity and insulin-stimulated lipogenesis by 50% in adipose tissue (Walton et al., 1987), as a result, increase the circulating insulin level in pigs. However, in Harter-Dennis' trial (1994), feeding NMA to female broilers did not produce any detectable effects on T3, T4, and insulin level. This inconsistency may be attributed to the species and the dose difference between two trials. Our preliminary experiment and numerous other researches indicated that NMA produces the various results at different doses (Harter-Dennis et al., 1994; Estienne et al., 1996, 1997, 1998).

As our knowledge, only few researches investigated the change of IGF-I level in pigs fed NMA. Estienne, et al. (1994) reported that, in broilers, NMA gavaged into the crop increase circulating IGF-I concentration. In current study, feeding NMA at a dose of 50 mg/kg to finishing pig significantly increase the IGF-I level in the serum, liver and muscle. We postulated that this increase is primarily caused by the stimulation of GH release in pituitary. Previous researches have shown that NMA could stimulate the GH secretion in pituitary via excitation of hypothalamus-pituitary axis. Nevertheless, in Harter-Dennis' trial (1994), no significant difference of IGF-I level was detected in NMA fed broiler.

Most recently, Estienne et al. (2000b) reported that

feeding doses of NMA ranging from 100 to 300 mg/kg had an overall negative effect on carcass yield characteristic in barrows, increasing backfat thickness at the 10th rib ($p<0.08$) and last rib ($p<0.03$), decreasing the lean percentage in the carcass ($p<0.05$). They attributed these results to the possible increase of somatostatin by NMA. In some experimental models, excitatory amino acids have been shown to stimulate not only GHRH secretion but also somatostatin release as well (Brann, 1995). Studies performed in primary cultures of rat fetal diencephalic neurons have shown that EAAs stimulate the release of SS (Tapia-Arancibia et al., 1988). Elevated levels of somatostatin may result in suppressed GH release, subsequent to increase adipose tissue deposition. It must be pointed out, however, that SS does not only simply inhibit GH secretion, but also plays a synchronizing facilitatory function of GHRH action (Willonghby et al., 1983). In addition, in our trial, although serum concentration of SS had an increase trend in NMA fed pigs, no significant difference was detected. Therefore, the inhibition of GH synthesis and secretion by elevated SS level due to NMA treatment was not observed in present trial.

In Harter-Dennis trial (1994), NMA produced a significant quadratic response in reducing ABF, linear and quadratic response in reducing percentage of shell fat in female broilers. Similarly, in present study, fat metabolic enzyme activities in subcutaneous adipose tissue were significantly changed in NMA fed pigs. The total and specific activities of MDH, G-6-PDH were significantly decreased with addition of NMA in diet. Meanwhile, the total activity of HSL was significantly increased in NMA fed pigs. Stimulation of GH release may contribute to the change of these enzyme activities. Magri et al. (1990) reported that the reduction in the lipogenic rate induced by GH was associated with a marked decline in the activity of several lipogenic enzymes: G-6-PDH (50% decrease), MDH (60% decrease), which diminished the rate of lipid synthesis. Our results further suggested that NMA seemingly increased the porcine lipid degradation, since total HSL activity was increased in NMA fed pigs. Alternatively, more evidences are needed before this conclusion can be drawn completely.

CONCLUSION

In summary, 50 mg/kg NMA had significant effect on growth associated hormone status in finishing pigs, and also dramatically changed the activity of several important fat metabolic enzymes, consequently, may cause a change of carcass characteristics in finishing pigs. Based on previous researches, different doses of dietary NMA caused various results in different animal species. Therefore, further scrutiny researches need to be done to elucidate the subtle effect of NMA.

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