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Evaluation of the Potential Role of Alpha-lipoic Acid with Regard to Health and Performance of Weanling Pigs

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Abstract: The objectives of this study were to: 1) determine the affect of supplemental lipoic acid (LA) on feed conversion and rate of gain on weanling pigs and 2) to evaluate the potential immunological benefits of supplementing piglet starter diets with lipoic acid. Twenty-one weaned gilts (d 21, 7 kg) were randomly allotted to three treatment groups: 1) Control, no supplemental LA (n = 7); 2) LA-8, orally supplemented with 8 mg kg^{-1} body weight of LA per day (n = 7); and 3) LA-15, orally supplemented with 15 mg kg^{-1} body weight of LA per day (n = 7). Pigs were individually penned and treatments were administered orally for 12 days. Feed intake and body weights were recorded from d 0 to d 12. On d 11, pigs were nonsurgically fitted with jugular cannulae. On d 12, all pigs received a dose of 150 ug kg^{-1} of lipopolysaccharide (LPS). Blood samples were collected at 15 min intervals for 30 min prior to the LPS challenge and for 180 min after LPS challenge to monitor serum cortisol, tumor necrosis factor-alpha (TNF-α), glucose and insulin. Additional whole blood samples were collected to determine total neutrophils and lymphocytes, as well as percentages. After the last blood sample was collected, all pigs were sacrificed for the collection of muscle and liver samples. Orally supplemented LA had no effect ($P > 0.05$) on average daily gain, average daily feed intake, or gain to feed ratios. No effect of LA was seen throughout the LPS challenge period ($P > 0.05$) on serum cortisol or tumor necrosis factor-α (TNF-α) concentrations. For serum glucose concentrations, differences among treatments were observed at -30 min pre-LPS such that serum glucose was lower ($P < 0.05$) in the LA-8 and LA-15 groups as compared to the LA-0 group. A LA treatment effect ($P < 0.05$) was observed between 45 and 165 min post-LPS, as LA-8 had higher serum glucose as compared to the LA-0 and LA-15 groups. From 120 until 165 min post-LPS, both LA-8 and LA-15 had higher serum glucose as compared to the LA-0 group ($P < 0.05$). Serum insulin concentrations did not differ ($P > 0.05$) among groups due to LA supplementation. There was a trend ($P < 0.15$) for the LA-15 group to have lower glycogen content in the muscle samples as compared to the LA-0 and LA-8 groups. Glycogen content in the liver was lower in the LA-0 group as compared to the LA-8 group. On d 12, prior to the LPS challenge, the percentage of neutrophils was greater and the percentage of lymphocytes was lower ($P < 0.05$) in the LA-15 group as compared to the LA-0 and LA-8 groups. Further research is needed to determine if supplemental LA may indeed be utilized as a beneficial immunomodulator in swine.

Key words: Pigs, dexamethasone, growth

Introduction

Performance losses during the early post-weaning period of swine production are one of the greatest challenges faced by pork producers. Piglets that maintain good health status early in life perform better during the growing and finishing phases of production and could potentially reach market weight in less time. This concept has led to the utilization of different production practices to help improve immune system function in piglets during this stressful time.

Two production practices widely used in the United States are segregated early weaning (SEW) and feeding subtherapeutic levels of antibiotics. Improved growth performance has been associated with SEW production, primarily due to removal of pigs from continuous exposure to antigenic challenges present in the sow herd. This management strategy has proven to be effective, however, due to an increase in labor and pen space requirements and problems arising from the piglet's immature digestive and immune systems, it can be problematic for producers. The practice of feeding subtherapeutic levels of antibiotics has also proven effective in improving growth performance, reducing mortality, morbidityand sub-clinical disease. Recently, worldwide attention has focused on media reports alleging the connection between the overuse of antibiotics in agriculture and the emergence and spread of antibiotic resistant bacteria. Due to this public concern, producers and scientists have been urged to find an alternative to feeding subtherapeutic levels of antibiotics.

Recently, it has been determined that antioxidants can play a role in decreasing the effects of disease in animals. Studies conducted using vitamin E or vitamin

C have produced positive results on immune function in many species (Bendich *et al.*, 1986; Kristensen *et al.*, 1986 and Webel *et al.*, 1998). These studies are limited by the physiological properties of vitamin E and C in that vitamin E is fat-soluble and vitamin C is water-soluble. An antioxidant that is both fat- and water-soluble is lipoic acid. Lipoic acid is an important cofactor in multienzyme complexes used for the production of energy and has been found to be a powerful antioxidant that also has the ability to recycle the antioxidants vitamin E and C. Utilizing lipoic acid as a dietary supplement to enhance immune function could enhance growth and health statusand could potentially. reduce the additional expense associated with dietary antibiotics.

This study proposed that lipoic acid used as a dietary supplement would enhance immune function and reduce economic losses due to poor performance. This hypothesis was developed due to the different functions of lipoic acid, which include serving as a cofactor in the multienzyme complexes used in the production of energy, enhancement of glucose uptake into cells via recruitment of the same transporters that insulin stimulates for glucose uptake, antioxidant activities of free radical scavengingand the ability to recycle essential antioxidants such as vitamin E and vitamin C.

The objectives of this study were to: 1) determine the affect of supplemental lipoic acid on feed conversion and rate of gain on weanling pigs and 2) to evaluate the potential immunological benefits of supplementing piglet starter diets with lipoic acid.

Materials and Methods

Animals: Gilts (21 days of age; n=21) weighing approximately 7 kg were weaned from their dams and moved from the University of Missouri swine farm to the University of Missouri Animal Science Research Center. Gilts were individually penned on a raised deck with plastic coated woven wire flooring and had *ad libitum* access to fresh water. Temperature was maintained in the thermoneutral zone for piglets of this size and age for the duration of the study. All animal procedures were reviewed and approved by the University of Missouri Institutional Animal Care and Use Committee.

Commercial hybrid gilts bred for high lean gain were fed a commercially available phase-one starter diet that consisted of at least 25% crude protein and other nutrients as described in Table 1. All diets were fed *ad libitum* from a self-feeder. Feed intake was recorded for a period of 10 days through d10 of the trial. Lipoic acid (LA; 99.9% pure; MTC Industries, Inc., Long Island, NY) was supplemented orally for a period of 10 days. Based on ingredients in the commercial ration, naturally occurring levels of LA in the feed were likely very low and therefore did impact the LA concentration supplemented.

Table 1: Composition of phase-one starter diet

Item	Calculated Composition, %
Crude Protein	≥ 25%
Lysine	1.8%
Crude Fat	≥ 6.5%
Crude Fiber	≤ 2.5%
Calcium	0.7-1.2%
Phosphorus	0.7%
Sodium Chloride	0.3-0.8%
Selenium	0.3 ppm
Zinc	2000 ppm
Carbadox	50 g T^{-1}

Gilts were randomly assigned to one of three supplemental treatments. The control group did not receive supplemental LA (LA-0), treatment group 1 was given 8 mg kg^{-1} body weight of LA per day (LA-8)and treatment group 2 was given 15 mg kg^{-1} of body weight of LA per day (LA-15). The oral doses were divided into two doses per day. The LA was measured and mixed into a semi-purified, high starch pig starter ration and fed orally to the gilts.

Immune Challenge: On d 0 of the trial (prior to lipoic acid supplementation), blood samples were collected using a jugular stick into a Vacutainer® brand blood collection tube (Becton Dickinson VACUTAINER systems; Franklin Lakes, New Jersey). On d 11 of trial, pigs were non-surgically fitted with indwelling jugular catheters in accordance with the procedure previously described by Carroll *et al.* (1999).

On d 12, feed was removed and the gilts were intravenously administered lipopolysaccharide (LPS; *Escherichia coli* serotype 0111:B4; Sigma L-2630; St. Louis, MO) at a dose of 150 μg kg^{-1} BW via the jugular catheter. Blood samples were collected at 15-minute increments for 30 min before and 180 min after the LPS was administered. Blood samples were allowed to set 1 h after collection at room temperatureand then refrigerated overnight. The following day, samples were centrifuged at 2400 rpm for 30 minutes at 4°C. Serum was collected and stored at −80°C until further analysis. Three additional blood samples were collected with sodium heparin as an anticoagulant (d 0 and d 12 at 0 min and 180 min post-LPS) to assess white blood cell counts.

Tissue Collection: Immediately after the last blood sample was collected at 180 min post-LPS, all pigs were sacrificed for tissue collection. Approximately 5 g of longissimus dorsi muscle, semimembranosus muscle and liver were collected, snap frozen in liquid

nitrogenand stored at −80°C.

Measurements: Feed additions were recorded daily. Feed intake, piglet weight gain and feed efficiency were calculated at the end of the 10-d test period. Serum parameters that were measured included cortisol, tumor necrosis factor-alpha (TNF-α), glucoseand insulin. Serum concentration of cortisol was determined using a Coat-a-Count assay kit (Diagnostic Products Corp.), which we have previously validated in our laboratory (Daniel *et al.*, 1999). Serum concentrations of TNF-α were determined utilizing a pig TNF-α ELISA kit as per the instructions of the manufacturer (Pierce-Endogen, Inc., Woburn, MA). Serum glucose concentrations were measured in duplicate using a commercially available colorimetric assay kit (Sigma Diagnostics, St. Louis, MO)and serum insulin concentrations were measured in duplicate using a commercially available radioimmunoassay kit (Linco Research, Inc, St. Charles, MO). Whole blood samples were sent to the University of Missouri, College of Veterinary Medicine diagnostic laboratory for white blood cell count, total and percentages of neutrophils and total and percentages of lymphocytes. Muscle and liver glycogen concentrations were measured using an acid hydrolysis reaction using the modified procedure of Keppler and Decker (1971). Muscle and liver tissue samples weighing 0.5 g were homogenized in ice-cold 0.6 N perchloric acid. The homogenate (200 μl) was reacted with 100 μl 1M potassium carbonate and 2.0 ml amuloglucosidase in acetate buffer. The samples were then incubated at 40°C for 2 hoursand the glucose concentrations were determined using a commercially available colormetric assay (Sigma, St. Louis, MO).

Analysis: Statistical analyses of growth, feed intakeand glycogen content data were performed using the PROC GLM procedure of SAS (SAS Inst. Inc., Cary, NC) using lipoic acid treatment as the source of variation for the model. Measurements taken over time were analyzed using PROC MIXED procedures of SAS specific for repeated measures using lipoic acid treatment, time during LPS challengeand time by treatment interaction as the source of variation for the model. Significant differences between treatment means were noted at $P<0.05$.

Results

Orally supplemented lipoic acid (LA) had no effect ($P>0.05$) on average daily gain, average daily feed intake, or gain to feed ratios as shown in Table 2. A time effect ($P<0.05$) was observed for serum cortisol concentrations (Fig. 1) such that serum cortisol increased after the LPS challenge in all treatment groups. Beginning at 15 min post-LPS, serum cortisol concentrations began to increase and remained elevated throughout the 180 min post-LPS challenge period ($P<0.05$). No effect of LA was seen throughout the LPS challenge period ($P>0.05$) on serum cortisol concentrations.

As with serum cortisol, a time effect ($P<0.05$) was observed for serum concentrations of tumor necrosis factor-α (TNF-α; Fig. 2) such that TNF-α concentrations increased after the LPS challenge in all treatment groups. Tumor necrosis factor-α concentrations increased from 30 min to 60 min post-LPS and then decreased through 180 min post-LPS to baseline levels for all treatment groups. While no overall treatment differences were observed, serum concentration of TNF-α were lower in the LA-15 treatment group at 60 min post-LPS as compared to the LA-0 and LA-8 treatment groups ($P>0.05$).

Serum glucose concentrations (Fig. 3) increased for all treatment groups from 0 min (immediately before LPS injection) to 45 min post-LPS and decreased from 45 min through 180 min post-LPS. Differences between treatments were observed at −30 min pre-LPS such that serum glucose concentrations were lower ($P<0.05$) in the LA-8 and LA-15 treatment groups as compared to the LA-0 treatment group. However, no differences ($P>0.05$) were observed at −15 min pre-LPS among treatment groups. A LA treatment effect ($P<0.05$) was observed between 45 and 165 min post-LPS, as LA-8 had higher serum glucose concentrations as compared to the LA-0 and LA-15 treatment groups. From 120 until 165 min post-LPS, both LA-8 and LA-15 had higher serum concentrations of glucose as compared to the LA-0 treatment group ($P<0.05$).

Serum insulin concentrations (Fig. 4) did not differ ($P>0.05$) between treatment groups during the pre-LPS sampling period. Following the LPS challenge, a time effect ($P<0.05$) was observed for serum insulin concentrations such that insulin concentrations fluctuated over time in all treatment groups. However there was no overall LA treatment effect on serum concentrations of insulin during a LPS challenge ($p>0.05$).

Glycogen content associated with the muscle and liver samples is presented in Table 3. For muscle samples of longissimus dorsi and semimembranosus taken after the LPS challenge, there was no difference ($P>0.05$) in glycogen content among the LA treatment groups. However, there was a trend ($P<0.15$) for the LA-15 treatment group to have lower glycogen content in the longissimus dorsi and the semimembranosus as compared to the LA-0 and LA-8 treatment groups. Glycogen content in the liver was lower in the LA-0

Table 2: Growth performance parameters of weanling pigs following 12 days of lipoic acid (LA) supplementation[a]

	LA-0	LA-8	LA-15	SEM	P-value
Final wt (kg)	11.8	11.5	12.0	0.71	0.879
ADG (kg)	0.43	0.40	0.44	0.03	0.684
ADFI (kg)	0.52	0.51	0.54	0.04	0.856
Gain:Feed	0.84	0.79	0.82	0.04	0.359

[a] All pigs were fed a commercially available starter phase-one diet. Pigs were supplemented 0 mg LA kg^{-1} BW d^{-1} (LA-0), 8 mg LA kg^{-1} BW d^{-1} (LA-8)and 15 mg LA kg^{-1} BW d^{-1} (LA-15) for 12 days. Each number represents the mean of seven pigs per treatment

Table 3: Muscle and liver glycogen content in weanling pigs following 12 days of lipoic acid (LA) supplementation and a 180 minute lipopolysaccharide challenge[a]

	LA-0	LA-8	LA-15	SEM	P-value
LDb (mg/g)	53.09	52.64	42.34	4.09	0.134
SMc. (mg/g)	52.21	54.26	43.15	4.29	0.142
Liver (mg/g)	11.64[d]	16.39[e]	12.26[de]	1.58	0.090

[a] Pigs were supplemented 0 mg LA kg^{-1} BW d^{-1} (LA-0), 8 mg LA kg^{-1} BW d^{-1} (LA-8)and 15 mg LA kg^{-1} BW d^{-1} (LA-15) for 12 days. Each number represents the mean of six pigs per treatment group. Glycogen concentration of muscle and liver was determined from tissue collected 180 min after being injected with a challenge dose (150 ug kg^{-1} BW) of lipopolysaccharide (LPS).

[b] Longissimus dorsi muscle (LD)

[c] Semimembranosus muscle (SM)

[de] Means lacking a common superscript in row differ ($P<0.05$)

Table 4: Neutrophil and lymphocyte percentages of total white blood cell count in weanling pigs before 12 days of supplementation with lipoic acid (LA), after supplementation with LAand 180 minutes after a lipopolysaccharide (LPS) challengea

	LA-0	LA-8	LA-15	SEM	P-value
Neutrophil (percents)[b]					
Basal	37.8	39.3	38.1	3.66	0.917
Pre-LPS	38.1[d]	41.6[d]	50.4[e]	3.01	0.019
Post-LPS	18.4	27.0	23.6	5.32	0.524
Lymphocyte (percents)[c]					
Basal	54.0	54.3	54.1	2.61	0.997
Pre-LPS	56.9[d]	52.4[d]	40.4[e]	3.57	0.007
Post-LPS	76.1	63.7	73.1	5.49	0.192

[a] Pigs were supplemented 0 mg LA kg^{-1} BW d^{-1} (LA-0), 8 mg LA kg^{-1} BW d^{-1} (LA-8)and 15 mg LA kg^{-1} BW d^{-1} (LA-15) for 12 days before challenge. Blood samples were collected on d 0 (prior to LA supplementation; Basal)and d 12 of trial before LPS challenge (Pre-LPS) and 180 minutes after LPS challenge (Post-LPS). Each number represents the mean of seven pigs per treatment.

[b] Changes in mean percentages of neutrophils are different from basal to pre-LPS ($P < 0.05$) for treatment LA-15 and from pre-LPS to Post-LPS ($P < 0.05$) for all treatment groups.

[c] Changes in mean percentages of lymphocytes are different from basal to pre-LPS ($P < 0.05$) for LA-15 and from pre-LPS to Post-LPS ($P < 0.05$) for all treatment groups.

[de] Means within a row lacking a common superscript differ ($P<0.05$).

treatment group as compared to the LA-8 treatment group, but did not differ ($P>0.05$) from the LA-15 treatment group.

Absolute neutrophil (Fig. 5) and lymphocyte (Fig. 6) counts were obtained on a per cubic mm basis. Basal neutrophil counts observed on d 0 of the study did not differ ($P>0.05$) among treatment groups prior to the initiation of LA supplementation (Basal). On d 12, after LA supplementation but prior to the LPS challenge (Pre-LPS), neutrophil counts were greatest ($P<0.05$) in the LA-15 treatment group as compared to the LA-0 and LA-8 treatment groups. Following the LPS challenge, neutrophil counts decreased ($P < 0.05$) in all treatment groups. However, there was no effect ($P>0.05$) of LA treatment on neutrophil counts following the LPS challenge.

Basal lymphocyte counts (Fig. 6) observed on d 0 of the study did not differ ($P>0.05$) among treatment

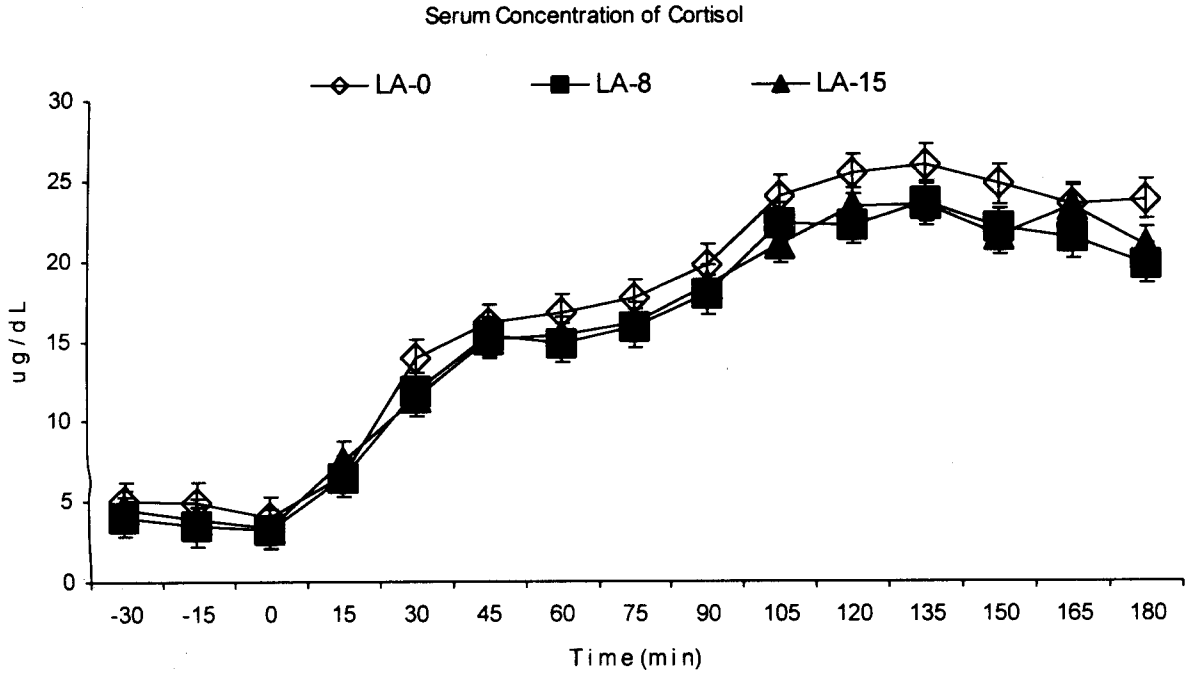

Fig. 1: Serum cortisol concentrations in 15 min increments from -30 minutes to 180 minutes after being injected (Time 0) with a dose (150 ug *kg* $^{-1}$ *BW) of lipopolysac*charide (LPS) following 12 days of lipoic acid (LA) supplementation. Pigs were supplemented 0 mg LA kg^{-1} BW d^{-1} (LA-0), 8 mg LA kg^{-1} BW d^{-1} (LA-8)and 15 mg LA kg^{-1} BW d^{-1} (LA-15) for 12 days before the LPS challenge. Data points represent means ± SEM of seven pigs/treatment group. An effect of LPS ($P<0.05$) was observed such that there was an increase in serum cortisol concentration over time in all treatment groups. There was no effect of LA treatment on serum cortisol concentration at any time during the LPS challenge ($P>0.05$)

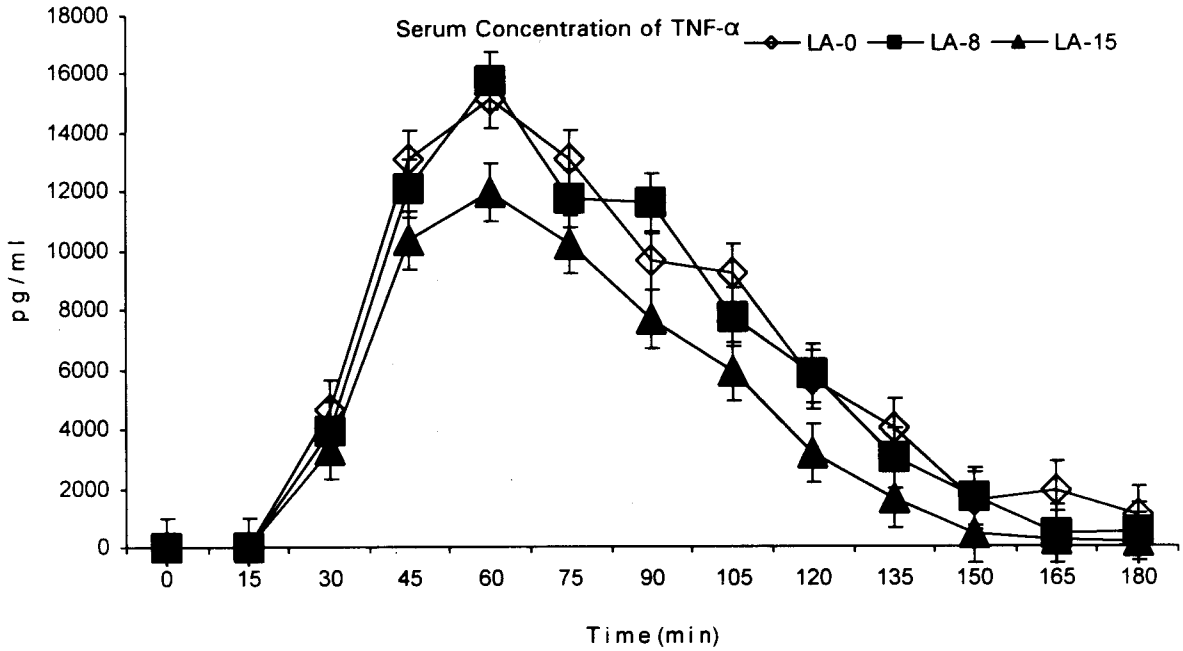

Fig. 2: Serum tumor necrosis factor-α (TNF-α) concentrations in 15 min increments from -30 minutes to 180 minutes after being injected (Time 0) with a dose (150 ug kg^{-1} BW) of lipopolysaccharide (LPS) following 12 days of lipoic acid (LA) supplementation. Pigs were supplemented 0 mg LA kg^{-1} BW d^{-1} (LA-0), 8 mg LA kg^{-1} BW d^{-1} (LA-8)and 15 mg LA kg^{-1} BW d^{-1} (LA-15) for 12 days before the LPS challenge. Data points represent means ± SEM of seven pigs/treatment group. An effect of LPS ($P<0.05$) was observed such that there was an increase in serum TNF-α concentrations over time in all treatment groups. There was no overall effect of LA treatment on serum TNF-α concentration during the LPS challenge ($P>0.05$)

groups prior to the initiation of LA supplementation (Basal). There were also no differences ($P>0.05$) observed in lymphocyte counts on d 12 after LA supplementation but prior to the LPS challenge (Pre-LPS). As with the neutrophil counts, lymphocyte counts were decreased ($P<0.05$) in all treatment groups following the LPS challenge.

To examine the overall profile change in the white blood cell population, we evaluated the percentage of neutrophils and lymphocytes (Table 4). The percentage of basal neutrophils and lymphocytes did not differ ($P>0.05$) among treatment groups. However, on d 12, prior to the LPS challenge, the percentage of neutrophils was greater ($P<0.05$) in the LA-15 treatment group as compared to the LA-0 and LA-8 treatment groups. During this same time period, the percentage of lymphocytes was lower ($P<0.05$) in the LA-15 treatment group as compared to the LA-0 and LA-8 treatment groups. After the LPS challenge, the percentage of neutrophils decreased ($P<0.05$) in all treatment groups, however there was no effect ($P > 0.05$) of LA supplementation. Likewise, the percentage of lymphocytes increased ($P<0.05$) in all treatment groups after the LPS challenge, but there was no effect ($P > 0.05$) of LA supplementation.

Discussion

Lipoic acid (LA) has been shown to increase uptake of glucose into muscle cells and adipocytes (Tsakiridis *et al.*, 1997) via an "insulin-like effect," which may cause a decrease in feed intake. High levels of circulating insulin are an indication of an increase in serum glucose concentration or that the host is in the "fed state." Oral supplementation of LA increases the concentration of free LA systemically. Since free LA has been reported to mimic insulin (Estrada *et al.*, 1996), the increased free LA could also generate the illusion of satiety and, therefore the subsequent reduction in intake. However, in the present study, we did not observe any differences in average daily feed intake or average daily gain. It's possible that the doses of LA

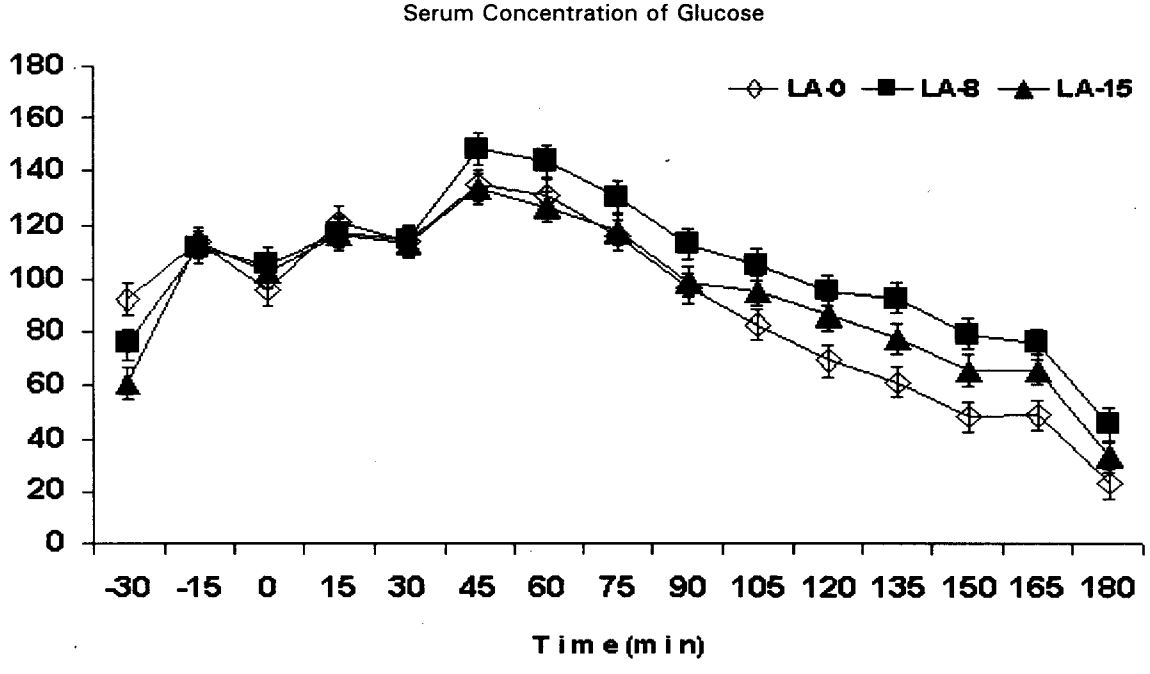

Fig. 3: Serum glucose concentrations in 15 min increments from -30 minutes to 180 minutes after being injected (Time 0) with a dose (150 ug/kg BW) of lipopolysaccharide (LPS) following 12 days of lipoic acid (LA) supplementation. Pigs were supplemented 0 mg LA kg^{-1} BW d^{-1} (LA-0), 8 mg LA kg^{-1} BW d^{-1} (LA-8)and 15 mg LA kg^{-1} BW d^{-1} (LA-15) for 12 days before the LPS challenge. Data points represent means ± SEM of seven pigs/treatment group. An effect of LPS ($P<0.05$) was observed such that there was an increase in serum glucose concentration over time in all treatment groups. At -30 minutes pre-LPS, differences were observed between all treatment groups ($P<0.05$). No treatment effect occurred for 45 min post-LPS ($P > 0.05$). However, from 45 to 165 min post-LPS, the LA-8 treatment group had higher concentrations of serum glucose ($P<0.05$) compared to the LA-0 and LA-15 treatment groups. From 120 to 165 min post-LPS, the LA-0 treatment group had lower concentrations of serum glucose ($P<0.05$) compared to the LA-8 and LA-15 treatment groups.

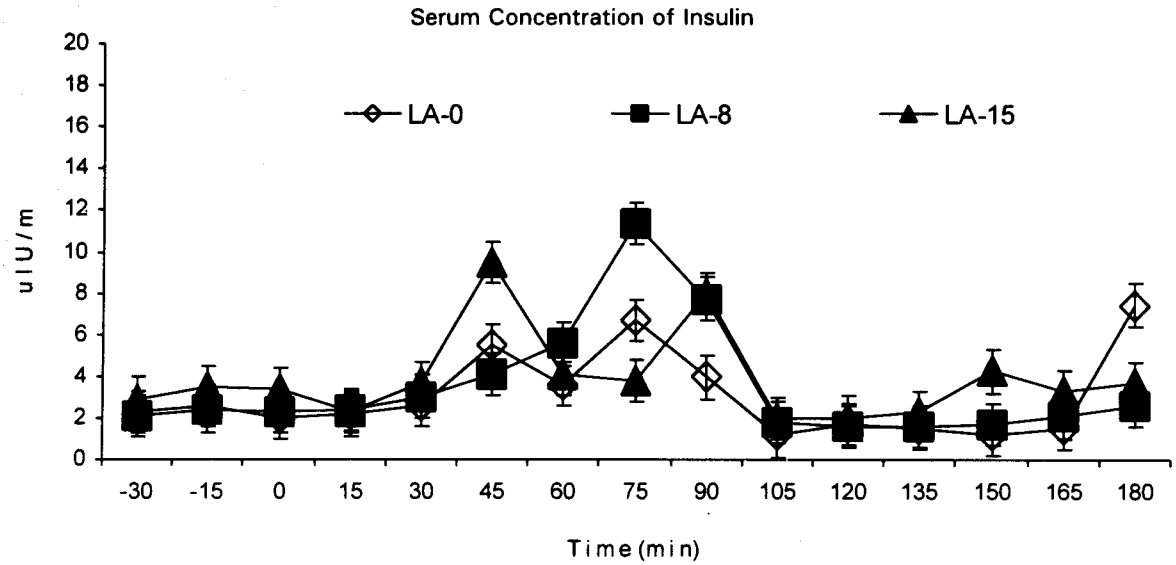

Fig. 4: Serum insulin concentrations in 15 min increments from -30 minutes to 180 minutes after being injected (Time 0) with a dose (150 ug/kg BW) of lipopolysaccharide (LPS) following 12 days of lipoic acid (LA) supplementation. Pigs were supplemented 0 mg LA kg^{-1} BW d^{-1} (LA-0), 8 mg LA kg^{-1} BW d^{-1} (LA-8)and 15 mg LA kg^{-1} BW d^{-1} (LA-15) for 12 days before the LPS challenge. Data points represent means + SEM of seven pigs/treatment group. An overall LPS effect ($P<0.05$) was observed such that there was an increase in serum insulin concentrations over time in all treatment groups. Lipoic acid supplementation had no effect on serum insulin concentrations during an LPS challenge ($P > 0.05$)

used in the present study were too low to alter feed intake even though the serum concentrations of glucose in the LA supplemented pigs were lower than that of the LA-0 pigs at -30 min prior to LPS challenge. In support of this idea is the fact that serum insulin concentrations were not altered in the LA supplemented pigs. While serum insulin concentrations were numerically higher in the LA-15 treatment group, an even higher dose of LA may be necessary to elicit sustained lower concentrations of glucose and subsequently higher concentrations of insulin. The effect of LPS challenge on serum glucose in the present study is consistent with research by Leininger *et al.* (2000) where serum concentrations of glucose decreased in all pigs after an LPS injection. Normal plasma glucose concentrations have been reported to be between 80 to 120 mg dl^{-1} (Guyton, 2000). Utilizing this range as normal, at time 0 in the present study, glucose concentrations would be considered normal in all treatment groups. It is interesting to note, however, that glucose concentrations observed at –30 min were well below normal for both LA-8 and LA-15 treatment groups. The -30 min sample was the first serum sample taken and the pigs were in a parasympathetic state and calm. Following the initial sample at -30 min, the presence of the researchers may have increased pig activity causing glucose to be released from the liver, thus eliminating any potential differences in basal glucose at time 0. Differences in serum glucose concentrations were also noted after the LPS challenge. Specifically, the LA-8 and LA-15 treatment groups had higher concentrations of serum glucose as compared to the LA-0 treatment group at several time points after the LPS challenge.

Lipoic acid has been shown to enhance uptake of glucose into cells via similar pathways used by insulin to stimulate glucose uptake. Approximately 80% of the total body glucose is stored as glycogen in the muscle. Despite this large storage capacity, free glucose cannot be released from muscle into circulation (via glycogenolysis) because muscle lacks the enzyme glucose-6-phosphatase. Therefore, it is the liver that serves as the primary source for maintenance of blood glucose levels. When the immune system is activated, stored glucose is released into the circulation to meet the increased energy demand.

While no differences were observed in total muscle glycogen content of the longissimus dorsi or semimembranosus muscles in the present study, differences in glycogen content of the liver were observed, even after fueling an immune challenge for 180 min. Glycogen content of the liver remained higher in the LA-8 treatment group as compared to the LA-0 control group after the LPS injection. Higher

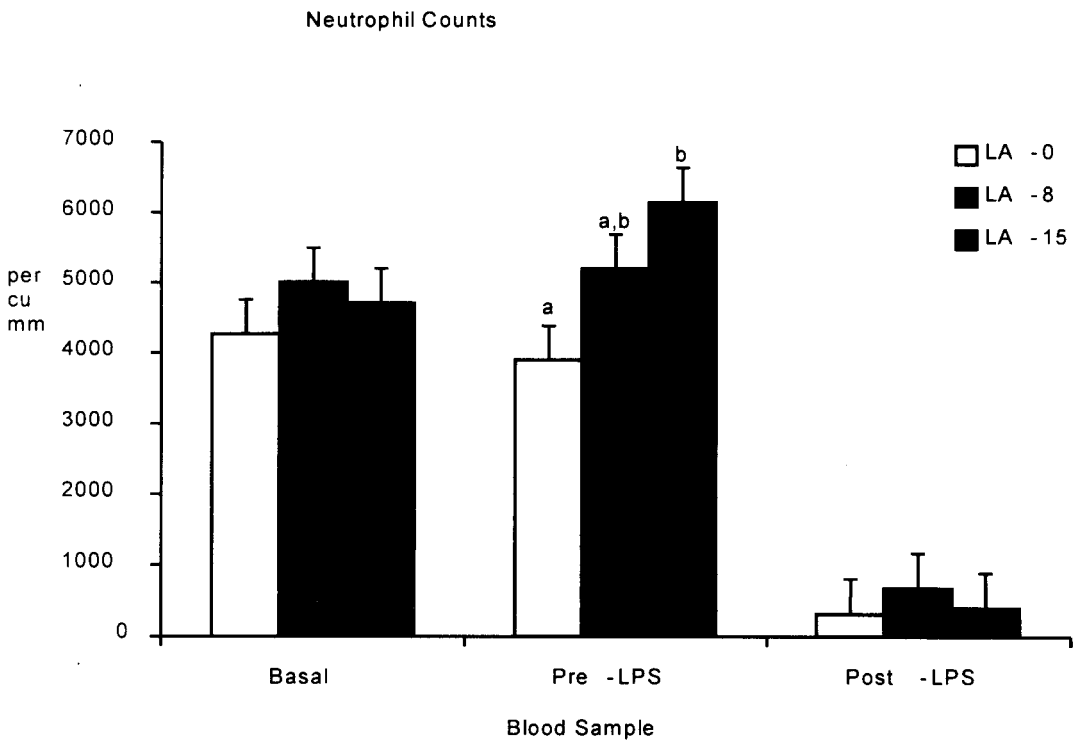

Fig. 5: Neutrophil counts (per cu. mm) in weanling pigs before 12 days of supplementation with lipoic acid (LA), after supplementation with LAand 180 minutes after a lipopolysaccharide (LPS; 150 ug/kg BW) challenge. Pigs were supplemented 0 mg LA kg^{-1} BW d^{-1} (LA-0), 8 mg LA kg^{-1} BW d^{-1} (LA-8)and 15 mg LA kg^{-1} BW d^{-1} (LA-15) for 12 days before challenge. Blood samples were collected on d 0 (prior to LA supplementation; Basal)and d 12 of trial before LPS challenge (Pre-LPS) and 180 minutes after LPS challenge (Post-LPS). Each bar represents the mean ± SEM of seven pigs. An overall effect of LPS was observed ($P<0.05$) such that there was a decrease in neutrophil counts between pre-LPS and post-LPS blood samples for all treatment groups. [ab] Means within same bleed time with different superscripts differ ($P<0.05$).

serum glucose levels were also observed during the immune challenge, whereby, the LA-8 piglets consistently had higher circulating glucose from 35 min to 180 min post-LPS injection. This would suggest that 12 d supplementation of LA resulted in compensation of glycogen in the liver, providing more readily available glucose to energize the immunological response.

For all treatment groups, serum concentrations of insulin appear to change between 45 and 90 min post-LPS. However, this change is not consistent between treatment groups as the LA-15 treatment group appears to spike at 45 and 90 min, with a third small spike at 150 min post-LPS. The LA-8 treatment group, on the other hand, has only one spike which occurred at 75 min post-LPS. It is difficult to determine the cause of these changes, as they do not seem to correlate with glucose concentrations. However, serum insulin concentrations may correlate with serum concentrations of TNF-α as TNF-α starts to increase at 30 minutes post-LPS. Insulin appears to follow at approximately 45 minutes post-LPS.

The effect of an LPS challenge on serum cortisol concentration is consistent with previous research from our laboratory as well as that of others (Webel *et al.*, 1997; Carroll *et al.*, 2002 and Frank *et al.*, 2003), as cortisol increases consistently through 180 min post-LPS. However, LA supplementation did not have a significant effect on serum cortisol concentrations following the LPS challenge. These results differ from the findings of Webel *et al.* (1998) where vitamin E decreased the serum cortisol concentration in young pigs during an LPS challenge and contradicts the idea that LA functions similarly to vitamin E during a stress challenge. However, again these results may reflect a need to increase the dose of LA as serum concentrations of cortisol were numerically lower in the LA supplemented pigs from 45 to 180 min post-LPS.

Tumor necrosis factor-α (TNF-α) is an inflammatory cytokine that is secreted by macrophages and

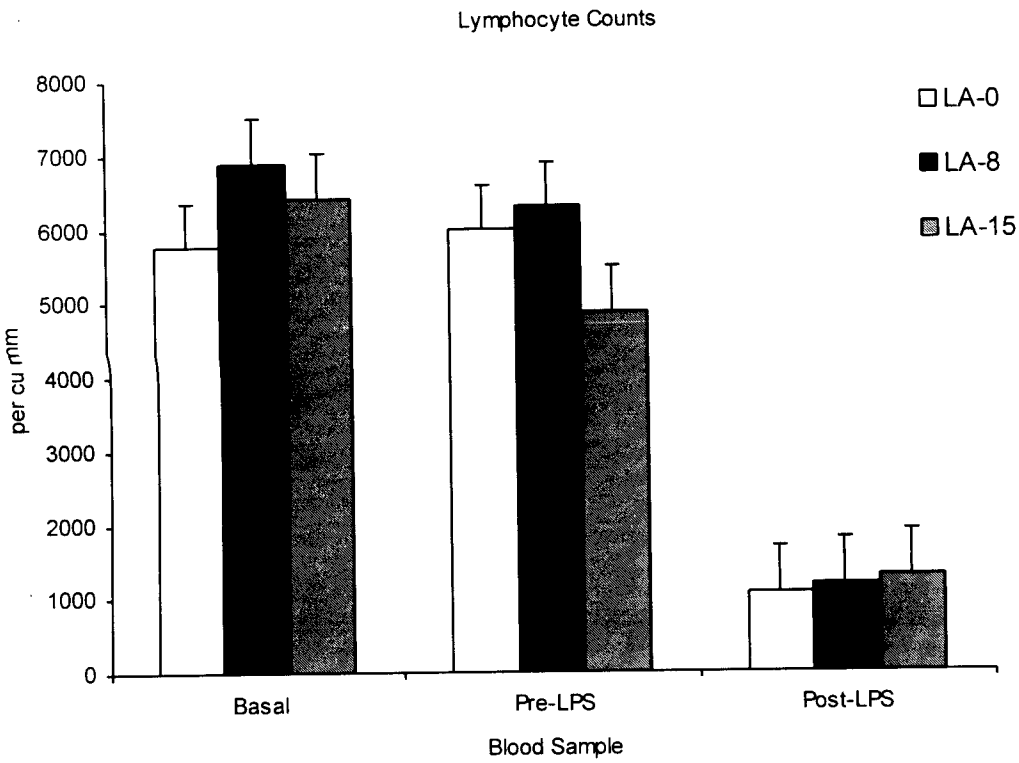

Fig. 6: Lymphocyte counts (per cu. mm) in weanling pigs before 12 days of supplementation with lipoic acid (LA), after supplementation with LAand 180 minutes after a lipopolysaccharide (LPS; 150 ug/kg BW) challenge. Pigs were supplemented 0 mg LA kg^{-1} BW d^{-1} (LA-0), 8 mg LA kg^{-1} BW d^{-1} (LA-8)and 15 mg LA kg^{-1} BW d^{-1} (LA-15) for 12 days before challenge. Blood samples were collected on d 0 (prior to LA supplementation; Basal)and d 12 of trial before LPS challenge (Pre-LPS) and 180 minutes after LPS challenge (Post-LPS). Each bar represents the mean ± SEM of seven pigs. An overall effect of LPS was observed ($P<0.05$) such that there was a decrease in lymphocyte counts between pre-LPS and post-LPS blood samples for all treatment groups

neutrophils, thereby increasing in concentration in the plasma during an immune challenge. Similar to this study, Webel *et al.*, (1997) demonstrated that TNF-α increased during an LPS challenge. In the present study, TNF-α increased in the serum from 15 to 60 min *post-LPSand* proceeded to decrease to near undetectable levels by 180 min post-LPS. While no significant differences were seen among treatment groups following the LPS challenge, it is interesting to note that the LA-15 treatment group had numerically lower concentrations of TNF-α. Our inability to detect a significant difference in serum TNF-α may be due to the small number of pigs per treatment group.

Neutrophil and lymphocyte total cell counts and percentages were measured before and after supplementation of LA for 12 daysand again after a 180 min LPS challenge. In pigs, the normal neutrophil percentage is approximately 38% and the normal lymphocyte percentage is approximately 53%. In the present study, these basal measurements were near the reported normal percentages. Notable differences were observed among treatment groups in the neutrophil and lymphocyte percentages prior to the LPS challenge. The percentage of neutrophils was higher in the LA-15 treatment group as compared with the LA-0 and LA-8 treatment groups. This pattern was also observed in the total neutrophil counts. While the percentage of lymphocytes was lower in the LA-15 treatment group as compared to the LA-0 and LA-8 treatment groups, this pattern was not observed in the total lymphocyte counts. However, differences in both neutrophil and lymphocyte total counts were observed from pre-LPS to post-LPS as both cell types decreased dramatically in the blood. Most likely, this decrease reflects an activation of the immune system. When cytokines are released, it causes a vasodilation of the blood vessels that allows for transport of lymphocytes and neutrophils into tissues. Also the endothelial cells

of the blood vessels become very "sticky" and hold these white blood cells to allow greater exposure to antigens present in the blood stream (Janeway *et al.*, 1999).

The increase in neutrophils in the LA-15 treatment group may be an effect of introducing an unknown factor (free LA) into the blood stream at a high enough level to cause recruitment of neutrophils, as they are the first defense against infection. The normal neutrophil count in pigs is between 4,000 and 8,000 per cubic mm and the normal lymphocyte count in pigs is between 5,800 and 12,000 per cubic mm. The number of neutrophils and lymphocytes observed for basal and pre-LPS sample times were within these normal ranges for all treatment groups. It should be noted that while a significant increase in neutrophil counts occurred between the basal and pre-LPS time points for the LA-15 treatment group, this increase was not large enough to cause levels to be above the reported normal range.

An increase in neutrophils in the blood could be beneficial during an immune response to help phagocytize antigens. While several indices measured in the present study indicate that LA has the potential to be used as an immunomodulator, further research needs to be conducted to determine if supplemental LA can provide adequate antioxidant activity which would be beneficial during an immune challenge. Based on the present results, it is difficult to determine if the observed effects were beneficial to the immune system. Follow up studies will need to evaluate growth and performance following the LPS challenge to adequately address this issue. Additionally, future research should also evaluate longer durations of LA supplementation, as well as higher doses of LA.

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