RESEARCH REPORT
ANIMAL SCIENCE

**Title:** Impact of Mitochondrially Targeted Novel Antioxidant on Pig Feed Efficiency - **NPB #11-126**

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**Industry Summary:** Oxidative stress is considered a potential cause of decreased feed efficiency in animals. Therefore, incorporating antioxidants into livestock feeds may improve feed efficiency by decreasing oxidative stress. This experiment was designed to determine if feed efficiency will be improved when a derivatized antioxidant that specifically targets the cellular mitochondria, the source of oxidative stress, is fed to growing pigs. Thirty barrows were fed a commercial swine diet for 6 weeks. Their body weight and feed intake was recorded weekly. Additionally, carcass composition was estimated by using ultrasound at the beginning and end of the experiment. During the six week feeding period, ten of the barrows were fed no antioxidants, ten were fed the underivatized antioxidant TBHQ, and ten were fed the derivatized antioxidant mitoTBHQ. When comparing the results of the control group with the TBHQ and mitoTBHQ groups, feeding antioxidants did not statistically improve average daily gain, feed efficiency, or lean tissue accretion. The inability of this experiment to detect differences these between treatment groups is likely because of the relatively small number of pigs in each treatment group. Although statistical differences were not detected, feeding mitoTBHQ increased average daily gain by 8.8% and improved feed efficiency by 6.8% compared with barrows in the control group. Future studies that incorporate greater numbers of pigs will likely be able to definitively establish the amount of improvement in growth rate and feed efficiency provided by mitochondrially targeted antioxidants.

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**Scientific Abstract:** Free radical production by the mitochondria and subsequent oxidative cellular damage may decrease mitochondrial function resulting in decreased growth rates and decreased feed efficiency. Therefore, mitigation of oxidative stress in growing animals is a potential target to improve feed efficiency in

livestock. Targeting antioxidant compounds to the mitochondria may increase the efficiency with which antioxidants decrease oxidative stress. Thirty weaned barrows (68.9 ± 7.4 kg) were used in a completely randomized design to test the effect of dietary tertiary butylhydroquinone (TBHQ) and the triphenylphosphonium derivatized mitochondrially targeted TBHQ (mitoTBHQ) on measures of growth and feed efficiency. Each barrow was assigned randomly to one of 3 treatment diets, Control, TBHQ (Control plus 8.546 mg/kg TBHQ per day), and mitoTBHQ (Control plus 30 mg/kg mitoTBHQ per day). Barrows were penned individually and fed for 6 wks. They had ad libitum access to water and a corn/soy commercial diet. Feed intake was recorded and barrows were weighed weekly. All barrows were evaluated for loin eye area and backfat thickness via ultrasound at the initiation of treatments and at the conclusion of the experiment. Ultrasound evaluation of the barrows was performed to estimate lean tissue accretion during the feeding phase. We hypothesized that an improved ADG and feed conversion ratio will be observed in barrows in the TBHQ group compared with the control barrows and that ADG and feed conversion ratio will be further improved in barrows in the mitoTBHQ group compared with the TBHQ group. Treatment did not alter final BW ($P$ = 0.8989), ADG ($P$ = 0.3374), feed conversion ratio ($P$ = 0.5330), or lean tissue accretion ($P$ = 0.1495). However, the ranking of all response variables measured were predicted by our hypothesis. Barrows in the mitoTBHQ group had greater final BW, greater lean tissue accretion, increased ADG, and improved feed conversion ratio compared with barrows in the TBHQ group. Likewise, Barrows in the TBHQ group showed improvement in every variable measured when compared with barrows in the Control group. Feeding mitoTBHQ resulted in an 8.8% improvement in ADG and a 6.8% improvement in feed conversion ratio compared with barrows in the Control group. Additional trials may be required to determine if mitochondrially targeted antioxidants can improve growth rate and feed efficiency adequately enough to make them profitable in the swine industry. Additional studies should incorporate greater numbers of experimental units and the determination of the optimal dosage of mitoTBHQ.

**Introduction:** Free radical production within the mitochondria resulting in oxidative damage to mitochondrial proteins has been thought to be a cause of decreased feed efficiency in animals. It has been proposed that antioxidants targeted to the mitochondria may decrease the amount of oxidative damage occurring as a result of free radical production. This concept has been proven in our laboratory by using mice and mitochondrially targeted vitamin E. Dietary antioxidants have been shown to decrease mitochondrial oxidative stress and relieve oxidative damage. Additionally, techniques have been developed to target antioxidants to the mitochondria that facilitate the efficient accumulation of exogenous antioxidant compounds within the mitochondria. Tertiary butylhydroquinone (TBHQ; Figure 1) and a triphenylphosphonium derivative of TBHQ (mitoTBHQ; Figure 2) were selected for this study to examine the effect of antioxidants and mitochondrially targeted antioxidants on the feed efficiency of growing barrows. The objective of this study was to determine the effect of feeding TBHQ and mitoTBHQ on barrow average daily gain, feed intake, feed efficiency, and carcass composition. We hypothesized that including TBHQ and mitoTBHQ in the diets of growing barrows will result in a decrease in reactive oxygen species generation and decreased oxidative damage to mitochondrial electron transport complexes and TCA cycle enzymes which would result in the measurable improvement in average daily gain and feed efficiency. Furthermore, we hypothesize that mitochondrial targeted antioxidants will improved average daily gain and feed efficiency to a greater degree than traditional antioxidants.

**Objectives:** Inefficiency in mitochondrial biogenesis and increased electron leakage resulting in reactive oxygen species production, oxidative stress, and inflammation may be a primary mediator of decreased feed efficiency in growing pigs. Our overall objective is to evaluate the effect of a novel antioxidant (mitoTBHQ) that is known to readily penetrate mitochondria for minimization of reactive oxygen species production on

mitochondrial function and feed efficiency. Our hypothesis is that decreasing the oxidative stress within the mitochondria of pigs with mitoTBHQ will improve feed efficiency and thus growth efficiency of pigs. We tested the derivatized antioxidant (common food additive) and with the control (no added antioxidant) in barrows.

**Materials and Methods:**

*Animals and Diets*

All procedures were approved by the Iowa State University Institutional Animal Care and Use Committee. Thirty crossbred barrows (68.9 ± 7.4 kg; PIC genetic lines) were housed in individual pens and had ad libitum access to water and a ground, commercial corn/soy based diet (Table 1). On wk 0, barrows were assigned randomly to 1 of 3 treatments for 6 wk. Control barrows received neither TBHQ nor mitoTBHQ. Barrows in the TBHQ group received 8.546 mg/kg BW per d. Barrows in the mitoTBHQ group received 30 mg/kg BW per d. To improve palatability and ensure consumption of the two compounds, TBHQ and mitoTBHQ were provided once daily concealed within a small amount of cookie dough. Barrows were weighed weekly and weekly feed intake was recorded. During wk 0 and wk 6, carcass ultrasound images of live animals were captured at the $10^{th}$ rib. These images were used to determine loin eye area and backfat thickness. The following equation was used to estimate pounds of lean tissue based on gender, live wt (pounds), backfat thickness (in), and loin eye area ($in^2$).

$$Pounds\ of\ lean\ tissue = (0.833) - \left(16.498 \times fat\ thickness\right) + \left(5.425 \times loin\ eye\ area\right) + (0.291 \times BW) - 0.534$$

*Statistical Analysis*

Data were analyzed using the MIXED procedure of SAS. Barrow was treated as the experimental unit and the model statement included the fixed effects of treatment with no random effects. Weekly BW data were analyzed as a repeated measure with fixed effects of treatment, wk, and treatment × wk interactions. Data are presented as least squares means ± SEM and differences were considered significant if $P < 0.05$, unless otherwise stated.

**Results:**

*Effect of TBHQ and mitoTBHQ on Feed Efficiency*

Neither TBHQ nor mitoTBHQ improved ADG or feed efficiency compared with Control barrows (Table 2). Weekly BW was not affected by treatment (Figure 3). Lean tissue gain as estimated by ultrasound measurement of backfat and loin eye area at wk 0 and wk 6 was not increased by feeding TBHQ or mitoTBHQ (Figure 4). Backfat thickness and loin eye area at wk 6 were not changed by feeding TBHQ or mitoTBHQ (Figure 5 and Figure 6, respectively).

Neither feeding TBHQ nor mitoTBHQ improved weight gain, feed efficiency, nor carcass composition of either antioxidant fed barrows compared with control barrows.

**Discussion:** Although the treatment group means for ADG, feed efficiency, BW, and lean tissue accretion did not differ, the treatment groups ranked in the order predicted by our hypothesis for each response variable. Barrows fed TBHQ had a greater ADG, BW at slaughter, lean tissue accretion, and feed efficiency compared with control barrows, and mitoTBHQ barrows ranked higher than TBHQ barrows for each response variable.

Feed conversion ratio and ADG improved in barrows fed mitoTBHQ. This response resulted in an improvement in ADG of 8.8% for barrows in the mitoTBHQ group compared with control barrows and an improvement in feed conversion rate of 6.8%.

The inability of this study to identify differences between treatments is likely the result of the relatively few pigs in each treatment group. Further research on the effect of these compounds on swine should utilize a greater number of experimental units. In addition, the bioavailability and potency of the novel mitoTBHQ has not been established. So, it is not certain if the optimal dose was provided in this study. Both TBHQ and mitoTBHQ were offered in equimolar dosages to allow us to evaluate their effectiveness relative to each other. The dosages that were selected were based on dosages that proved to be effective in previous studies using vitamin E and mito vitamin E. It is likely that identification of an optimal dosage of mitoTBHQ also could yield greater improvements in growth and feed efficiency.

Whereas this project did not identify a statistical improvement in growth or feed efficiency, it does indicate that further research utilizing more experimental units or the identification of an optimal dosage of mitoTBHQ may yield results with a detectable and significant improvement in growth rate and feed efficiency.

Table 1. Diet composition[a]

| Ingredient | Percentage |
|---|---|
| Corn | 60.6 |
| Soybean meal | 17.1 |
| Dried distillers grains | 20.00 |
| Vitamin and mineral premix | 2.0 |
| Lysine | 0.31 |
| Threonine | 0.0004 |
| Copper | 0.05 |

[a]As-fed basis

Table 2. Effects of TBHQ and mitoTBHQ on BW, feed intake, ADG, and feed conversion ratio

| | Treatment | | | | |
|---|---|---|---|---|---|
| Item | Control | TBHQ | mitoTBHQ | SEM | *P*-Value |
| Initial BW, kg | 67.43 | 68.8 | 68.98 | 2.59 | 0.8989 |
| Final BW, kg | 103.38 | 106.99 | 107.85 | 2.53 | 0.4325 |
| Feed intake, kg/d[a] | 2.36 | 2.50 | 2.39 | 0.100 | 0.5740 |
| ADG, kg | 0.844 | 0.909 | 0.918 | 0.038 | 0.3374 |
| Feed conversion ratio[b] | 0.363 | 0.365 | 0.388 | 0.018 | 0.5330 |

[a] Feed intake is reported on as-fed basis.

[b] Feed conversion ratio is calculated as average daily gain divided by average daily feed intake.

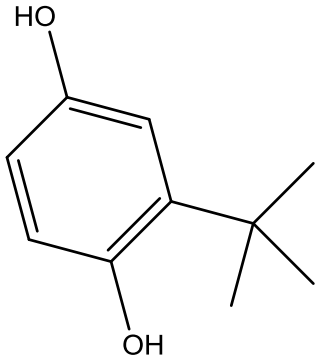


Figure 1. Structure of tertiary butylhydroquinone (TBHQ).

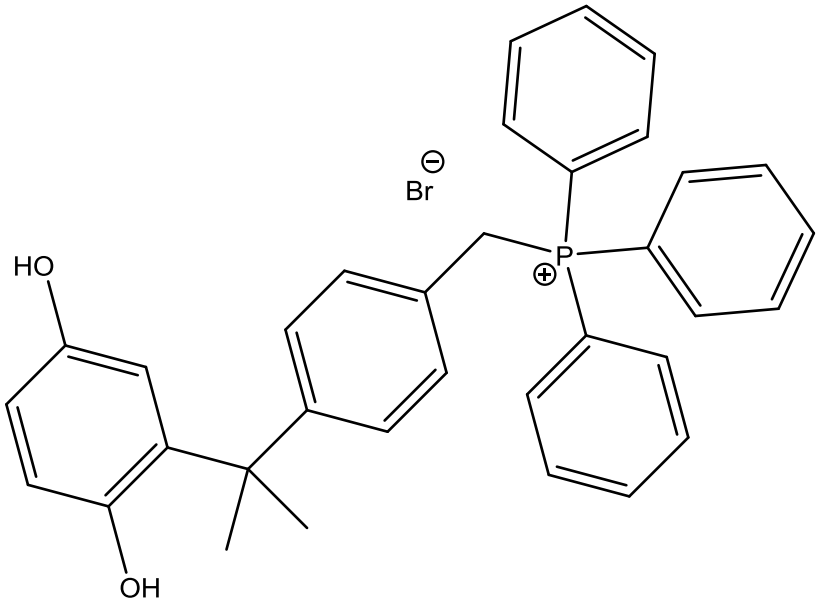


Figure 2. Structure of mitochondrially targeted tertiary butylhydroquinone (mitoTBHQ).

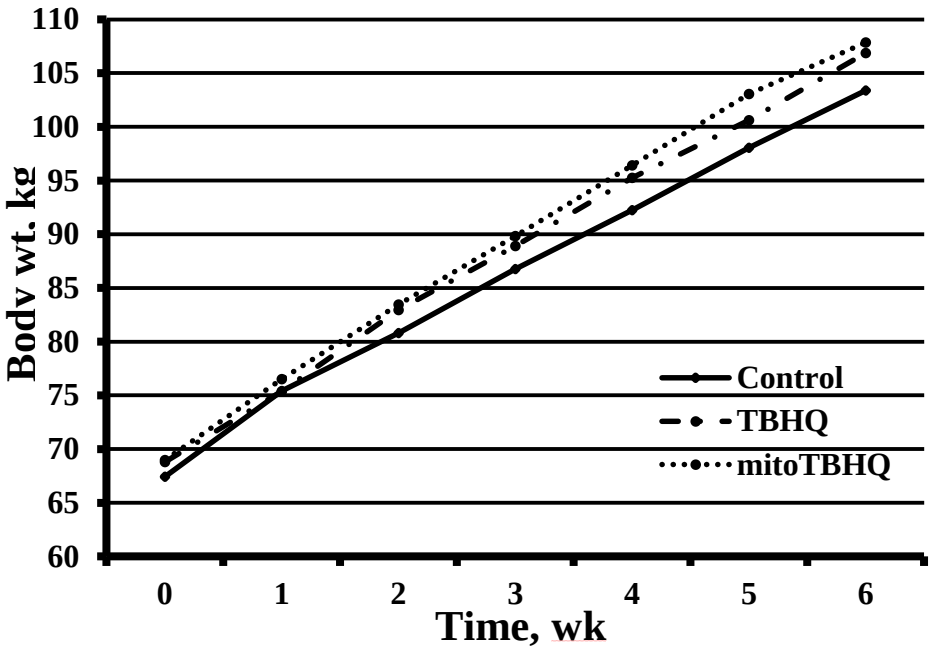


Figure 3. Weekly BW of barrows not fed antioxidants, barrows fed 8.546 mg/kg BW per day of TBHQ, and barrows fed 30.0 mg/kg BW per day of mitoTBHQ. Significance of treatment and treatment × wk interaction are $P = 0.6243$ and $P = 0.7964$, respectively.

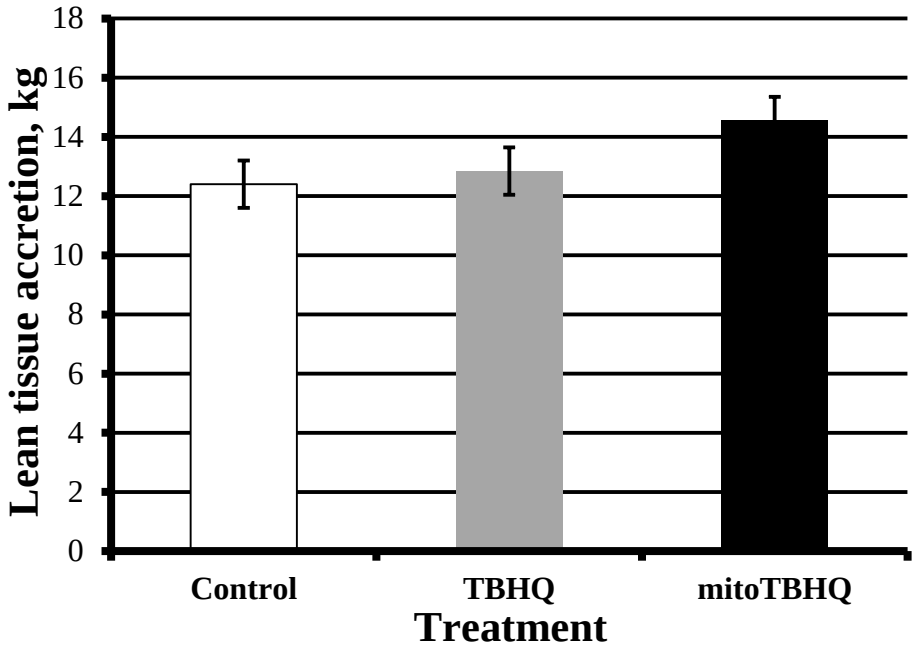


Figure 4. Estimated lean tissue accretion of barrows not fed antioxidants, barrows fed 8.546 mg/kg BW per day of TBHQ, and barrows fed 30.0 mg/kg BW per day of mitoTBHQ. Estimates based on ultrasound images of live animals. $P = 0.1495$.

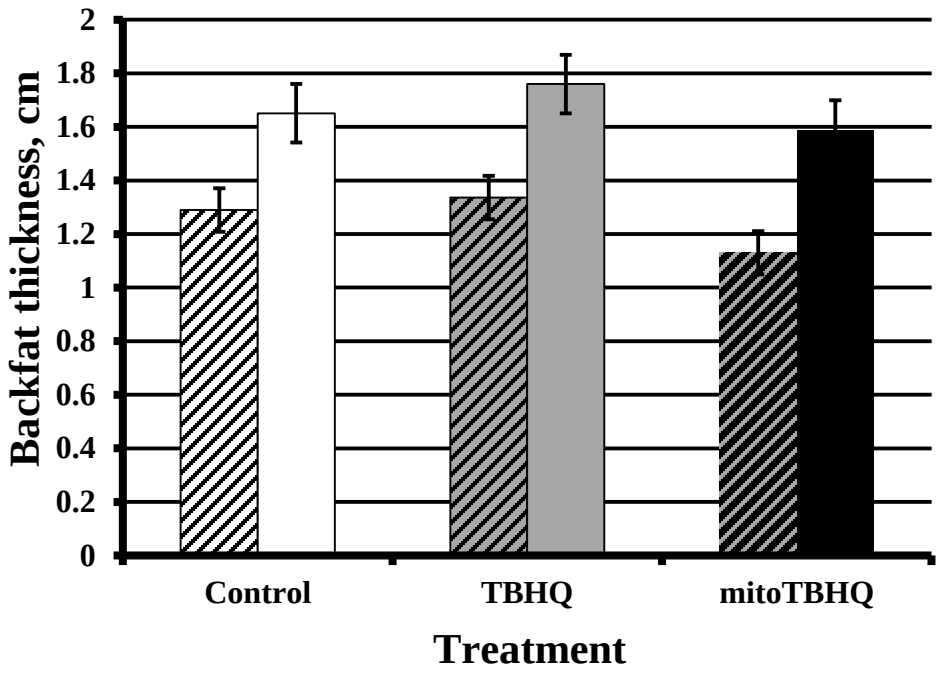


Figure 5. Backfat thickness on wk 0 and wk 6 of barrows not fed antioxidants, barrows fed 8.546 mg/kg BW per day of TBHQ, and barrows fed 30.0 mg/kg BW per day of mitoTBHQ. Hashed bars represent backfat thickness at wk 0. Solid bars represent backfat thickness at wk 6. $P = 0.5448$.

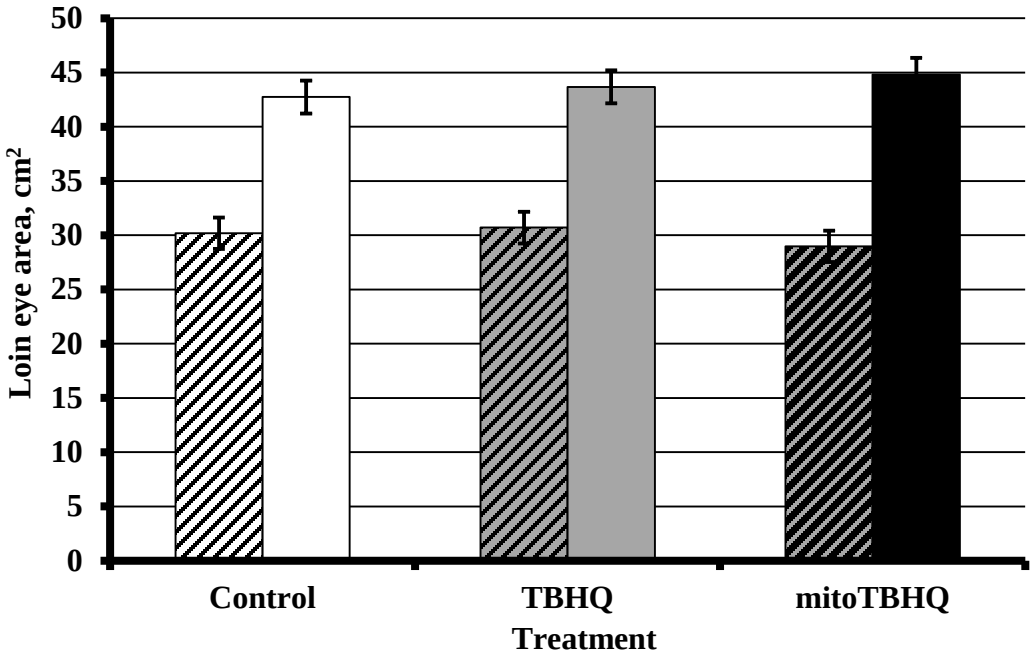


Figure 6. Loin eye area on wk 0 and wk 6 of barrows not fed antioxidants, barrows fed 8.546 mg/kg BW per day of TBHQ, and barrows fed 30.0 mg/kg BW per day of mitoTBHQ. Hashed bars represent backfat thickness at wk 0. Solid bars represent backfat thickness at wk 6. *P* = 0.6294.