

**Stress Tolerance and Immunocompetence of Sunshine Bass:
Use of Natural Source Vitamin E as a Nutraceutical in Aquaculture**

2nd and Final Report, June 2005-June 2006 (Project Year 2)

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Abstract

Our purpose was to investigate the use of d-Alpha® Natural Source Vitamin E (d-alpha tocopheryl acetate) in prepared diets for sunshine bass to enhance immunity and reduce stress-related mortality. Sunshine bass are well-suited to commercial culture, however, the industry is limited by the immunosuppressive consequences of stress. Administered at higher dosages, the properties of d-Alpha® may stimulate the immune system and compensate for the negative effects of stress. To fully investigate the potential of d-Alpha® as a nutraceutical supplement for sunshine bass, five objectives were developed: 1) determine the vitamin E requirement of sunshine bass as met by d-Alpha®, 2) evaluate the effects of nutraceutical-level supplementation with d-Alpha® on stress tolerance and nonspecific immunity of stressed sunshine bass, 3) evaluate nutraceutical effects of d-Alpha® supplementation in a disease challenge, 4) evaluate the effect of d-Alpha® supplementation on tissue fatty acid (FA) profiles and lipid metabolism during exertion and stress, and 5) conduct cost-effectiveness analyses of d-Alpha® as a nutraceutical feed supplement aquaculture feeds.

We determined the vitamin E requirement of sunshine bass can be met by 22 mg d-Alpha®/kg feed, less than the requirement reported for synthetic vitamin E (SYNE, Objective 1). Although no significant effects on stress tolerance were observed, increasing dietary d-Alpha® to roughly five times (5X) the minimum dietary requirement was associated with subtle modulatory influence on nonspecific immunocompetency (Objective 2). Although stress was associated with reduced fillet oxidative stability, increased dietary provision of d-Alpha® resulted in dramatic increases in fillet tocopherol content and improved post-slaughter stability. We subsequently compared requisite and 5X levels of SYNE and d-Alpha® during disease challenge of sunshine bass. Based on pathogen burden and clinical measures of nonspecific immunity, we concluded the immunomodulatory influence of d-Alpha® is unique and superior relative to SYNE with respect to nonspecific mechanisms of disease resistance (Objective 3). Further, super-requirement dietary levels of d-Alpha® may enhance immunocompetency of sunshine bass during infection and aid in pathogen clearance. Super-requirement levels of d-Alpha® were associated with increased energy mobilization during exertion of sunshine bass, potentially via optimization of adipose phospholipase activity and/or alterations in prostaglandin concentrations (Objective 4). Lipid metabolism was not significantly affected in this trial, however, increasing d-Alpha® was associated with beneficial shifts in fillet FA composition. Lack of direct, cause-effect relationships between dietary d-Alpha® content and measures of mortality/recovery due to disease precluded exhaustive cost-effectiveness analysis (Objective 5). However, we have demonstrated that d-Alpha® is more biologically potent than SYNE in meeting minimum dietary requirements of sunshine bass. Moreover, when provided at super-requirement levels, d-Alpha® consistently confers benefit beyond that achieved with SYNE. Currently, d-Alpha® is marketed at equivalent cost per unit vitamin E activity to SYNE. Given the increased potency and unique characteristics of d-Alpha®, nutraceutical application of this product may result in decreased cost of production (e.g., reduced feed cost, occurrence of disease) and increased product value (e.g., enhanced fillet tocopherol content, improved fillet stability) in sunshine bass aquaculture.

Keywords: sunshine bass, *Morone* spp., nutrition, vitamin E, nutraceutical

Introduction

Aquaculture is a rapidly expanding industry, particularly in the United States, with the government calling for an approximate five-fold increase in aquaculture production by the year 2025 (DOC 1999). In response, the industry is expanding the volume as well as the scope of production, investigating the viability of commercial production of other species. One of these developing industries is the production of sunshine bass, the result of crossing white bass *Morone chrysops* and striped bass *M. saxatilis*. Although sunshine bass are in many ways ideal for commercial production, practical culture of these fish is plagued by their susceptibility to stress and disease. Many necessary culture practices stress the fish, often resulting in infection and/or mortality. Stress-related complications are a limiting factor in sunshine bass production, and it is clear that if this industry is to flourish, these problems must be overcome.

Common culture procedures such as handling and transport can cause stress, as can exposure to reduced water quality. Although certain measures may be taken to reduce stress (e.g. adequate acclimation time, careful monitoring of water chemistry, handling fish only when absolutely necessary), it cannot be eliminated. Stressor exposure has been linked to impaired immune function in numerous fish species; the physiological consequences of stress, primarily deviations from bioenergetic homeostasis, have been implicated as causative factors underlying chronically suppressed immunity among cultured species (Tort et al. 2004)

Since stress cannot be entirely avoided in aquaculture, the only remaining option available to aquaculturists is to compensate for its effects. The specific thrust of the present research was to evaluate compensatory nutritional stimulation of the immune system to mitigate the effects of stress in sunshine bass. As a required micronutrient and the primary lipid-soluble antioxidant in living systems, vitamin E has long been associated with maintaining health and immunocompetency. In addition to reduced growth, abnormal pigmentation, liver necrosis, and decreased tissue stores, vitamin E deficiency in fishes is often typified by impaired immune function (Blazer 1992). Vitamin E supplementation has been shown, in a variety of species, to improve the function of phagocytes, stimulate the production of lymphocytes and protect existing lymphocytes, increase cell proliferation in immunopoietic organs and regulate the production of immune-response hormones (Blazer 1992). Meeting minimum dietary requirements is critical for ensuring proper immune function, however, research indicates current dietary recommendations may be insufficient to precipitate maximum immune function (Meydani and Beharka 1998). Moreover, as nutritionists forego collective descriptions of vitamin E and begin to address the micronutrient in terms of specific vitamers (α , β , γ , and δ tocopherols and tocotrienols), their stereoisomers (8 possible stereoisomeric configurations per isomer), and the relative contributions of these compounds to meeting dietary requirements, the definition of vitamin E's function and absolute requirements are being rapidly redefined.

Because of its immunomodulatory function and safety at high dosages, vitamin E is an ideal candidate for nutraceutical application. All E vitamers are roughly equivalent in terms of antioxidant potential (Zingg and Azzi 2004), however, α -tocopherol is

acknowledged as having the greatest biological activity (Hosomi et al. 1997). Like other E vitamers, the structure of α -tocopherol includes three chiral centers, allowing for eight possible stereoisomeric configurations. Of these stereoisomers, RRR- α -tocopherol has the greatest potency in vivo (Burton et al. 1998; Brigelius-Flohé and Traber 1999) due to a superior affinity of hepatic α -tocopherol transport protein for this stereoisomer (Stone and Papas 2003).

Human nutritional studies have established the potency of RRR- α -tocopherol as being roughly twice that of all-rac- α -tocopherol, a synthetically-prepared racemic mixture containing equal amounts of RRR- α -tocopherol and its racemates (Stone and Papas 2003). Despite the greater efficacy of RRR- α -tocopherol, animal feed manufacturers typically use synthetic vitamin E (SYNE, all-rac- α -tocopheryl acetate) to meet the vitamin E requirement of livestock. d-Alpha® natural source vitamin E (NSVE) is a purified source of RRR- α -tocopherol (as RRR- α -tocopheryl acetate), and is purported to be a superior alternative to SYNE for meeting dietary requirements of humans and livestock. NSVE may also be superior to SYNE in enhancing the immunocompetence and performance of aquatic livestock, however, these effects had not previously been addressed in the context of aquaculture nutrition.

To fully investigate the potential of d-Alpha® as a nutraceutical feed additive for the culture of sunshine bass, five objectives were defined.

1. Determine the nutritional requirement of sunshine bass for d-Alpha®, using production performance- and non-performance-based indicators of hypo- and hypervitaminosis.
2. Examine the effects of nutraceutical-level supplementation with d-Alpha® on stress tolerance and nonspecific immunity of stressed sunshine bass, and identify a dietary concentration of d-Alpha® that optimizes those parameters.
3. Compare the optimal diet(s) identified in Objective 2 to an equivalent synthetic vitamin E-based diets in a disease challenge experiment.
4. Examine the effects of nutraceutical-level d-Alpha® supplementation on tissue fatty acid profiles and lipid metabolism during exertion.
5. Conduct cost-effectiveness analyses of d-Alpha® nutraceutical application and using d-Alpha® in prepared feeds for sunshine bass.

Narrative Report, Year 1.

A 12-week feeding trial associated with Objective 1 was conducted early in Year 1 of the project. Based on our results, we determined the vitamin E requirement of sunshine bass can be met by 22 mg d-Alpha®/kg feed. The results of this Objective were presented at a midyear meeting with Mamduh Sifri, David Holzgraefe, and Mark Franklin of ADM's Aquaculture Task Force in October 2004, and formally to the Task Force in May of 2005. The results were also presented at the Annual Meeting of the American Fisheries Society in August 2004, and Aquaculture America 2005, and were recently published in the North American Journal of Aquaculture (Trushenski, J.T., and C.C. Kohler. 2006. Evaluation of natural source vitamin E, RRR-alpha-tocopheryl acetate, as a micronutrient in sunshine bass feed. North American Journal of Aquaculture 68:186-191).

Following the completion of Objective 1, a second feeding trial was initiated in January of 2005 to begin Objective 2. At the end of the feeding trial, experimental stressors were applied as delineated in the original proposal and all data for Objective 2 were successfully collected. We observed no immediate immunosuppressive effects of stress, however, stressor exposure resulted in increased vulnerability of fillet tissue to oxidative degradation. Increasing dietary NSVE was associated with increased resistance to oxidation of fillet tissue during storage and increased performance in humoral immunocompetence, suggesting NSVE possesses some ability to modulate performance in these response categories. Fillet incorporation of tocopherols was dramatically higher among fish fed increased levels of NSVE, and diet-to-fillet transfer rates observed were substantially greater than those reported for SYNE in other species. Maximal modulatory response was associated with a dietary concentration representing approximately 5 times (5X) the previously determined requirement. Preliminary results were presented to the ADM Task Force in May 2005 at the meeting previously mentioned. Final results were presented to the ADM Task Force in October 2005, at the 2005 Annual Meeting of the American Fisheries Society, Aquaculture America 2006, and AQUA 2006. A manuscript has been prepared and will be submitted for review, likely to Aquaculture Nutrition, in the near future.

Narrative Report, Year 2.

Objective 3 was completed in April of 2006. Diets containing requisite or 5X levels of SYNE or NSVE were compared in a stress and disease challenge (*Flavobacterium columnare*) experiment. Although stimulatory effects were not observed under normal culture conditions, super-requirement supplementation with NSVE compensated for stress and disease challenge-related reductions in immunocompetency of sunshine bass. Stressor exposure generally resulted in depressed immunocompetency, however, the magnitude of immunosuppression was attenuated in some respects among fish fed the NSVE-based diets, particularly those supplemented at super-requirement levels. The overall effect of stress on pathogen exclusion and/or clearance of sunshine bass was evidenced by greater systemic pathogen burden associated with stress-exposed groups. Cumulative dietary impact on resistance of sunshine bass to *F. columnare* is somewhat equivocal. Blood densities of *F. columnare* were lower among SYNE-fed fish within pathogen-exposed, non-stressed groups. When challenged with stress and pathogen exposure, 1X and 5X NSVE groups carried lower *F. columnare* loads than the 1X SYNE group, but bacterial densities were lowest among the 5X SYNE group. This would suggest greater resistance or pathogen clearance among fish fed elevated levels of SYNE, however, this was not clearly supported by the clinical measures of immunocompetency employed in the present work. We concluded the immunomodulatory influence of NSVE is unique and superior relative to SYNE with respect to nonspecific mechanisms of disease resistance. Further, super-requirement dietary levels of NSVE may enhance immunocompetency of sunshine bass during infection and aid in pathogen clearance. These results will be presented during the next ADM Task Force meeting and at the upcoming AQUA 2007 conference. A manuscript based on this project is currently in development.

Objective 4 was completed in August of 2005. Forced exertion was observed to down-regulate adipose phospholipase A₂ (cPLA₂) activity and affect resultant prostaglandin concentrations cPLA₂ activity and prostaglandin concentrations, although these effects were attenuated by an opposing effect of increasing NSVE. Super-requirement levels of d-Alpha® were also associated with increased energy mobilization during exertion of sunshine bass, potentially via optimization of adipose phospholipase activity and/or alterations in prostaglandin concentrations. Lipid metabolism was not significantly affected in this trial, however, increasing d-Alpha® was associated with beneficial shifts in fillet FA composition. Preliminary results were presented to the ADM Task Force in October 2005; final results were presented at Aquaculture America 2006 and AQUA 2006. A manuscript based on this project is currently in development.

Lack of direct, cause-effect relationships between dietary d-Alpha® content and measures of mortality/recovery due to disease precluded exhaustive cost-effectiveness analysis for Objective 5. However, we have demonstrated that d-Alpha® is more biologically potent than SYNE in meeting minimum dietary requirements of sunshine bass. Moreover, when provided at super-requirement levels, d-Alpha® consistently confers benefit beyond that achieved with SYNE. Currently, d-Alpha® is marketed at equivalent cost per unit vitamin E activity to SYNE. Given the increased potency and unique characteristics of d-Alpha®, nutraceutical application of this product may result in decreased cost of production (e.g., reduced feed cost, occurrence of disease) and increased product value (e.g., enhanced fillet tocopherol content, improved fillet stability) in sunshine bass aquaculture.

Potential Applications or Benefits

Natural source vitamin E has not been widely researched in animal nutrition, and the present work is the first to directly evaluate the relative nutraceutical potential of NSVE in an aquaculture species. We have conducted numerous trials with d-Alpha®, and have established a record of rigorous scientific assessment of this product. In addition to the scientific advancement our results represent, our research has added to the field of nutraceutical research in aquaculture. The project has generated a substantial amount of relevant data for Archer Daniels Midland Company (ADM, industry partner and producer of d-Alpha®) as well as garnered national and international interest among academic and industry representatives. We have successfully described the beneficial attributes of d-Alpha®, and have laid the foundation for implementation of this innovative product as a nutraceutical in aquafeeds.

Lay Summary

Stress results in a higher incidence of disease among livestock. Although stress cannot be removed from livestock production, its effects can be reduced through stimulation of the immune system. One means of increasing disease resistance is through the use of nutraceuticals, or dietary components that enhance immune function. These compounds are common in human nutrition (e.g. echinacea, ginseng, ginkgo, various antioxidants, etc.), and are beginning to be investigated in animal nutrition as well. Nutraceuticals are particularly appropriate for the aquaculture because contact with

livestock is essentially limited to feeding, and stress-related disease and losses remains a significant problem for this agricultural industry.

We investigated natural source vitamin E as a nutraceutical for use in sunshine bass (male striped bass crossed with female white bass) feeds. Although vitamin E is an essential nutrient required by all animals, at higher levels above the nutritional requirement, it has been shown to stimulate the immune system of animals, including fish. Natural source vitamin E is a specific chemical form isolated from oilseeds such as soybeans which has a greater potency than the synthetic form typically used in animal feeds. We hypothesized natural source vitamin E would have greater nutraceutical potential due to its greater biological activity. We found the nutritional vitamin E requirement of sunshine bass can be met by lower levels of natural source vitamin E compared to the synthetic form. Subsequent studies based on this initial finding indicated increasing the dietary concentration of natural source vitamin E maintained, or in some cases increased, disease resistance in sunshine bass. Additionally, use of natural source vitamin E may enhance the quality of aquaculture products by increasing fillet vitamin E content and shelf-life. In short, use of natural source vitamin E, particularly at higher dietary concentrations, is beneficial to aquatic livestock and can enhance product value.

International Implications

Sunshine bass are a primary U.S. aquaculture species, however our results may be applicable to other aquaculture species raised throughout the world. Along these lines, an Australian research team has expressed interest in developing similar applications of d-Alpha® in tuna aquaculture, and Ms. Trushenski is currently in contact with this team to develop potential applications. ADM, a multi-national company, will benefit from the scientific information provided by the project and will likely utilize the information we have provided in application and sales of d-Alpha®.

Media Coverage

In addition to the online announcements/articles published by Illinois-Indiana and National Sea Grant Offices, the fellowship program and this project has been featured in "Perspectives", a publication of Southern Illinois University Carbondale covering excellence in research (hard copy included). An article also appeared on the SIUC news website (hard copy included). The project was also featured multiple times on broadcasts of SIUC radio.

Partnerships

The project has continued reinforce the research collaboration between ADM and the Fisheries and Illinois Aquaculture Center. The specific outcomes of this project, as well as the collaborative environment it fosters, have led to several additional projects currently in development to evaluate application of other ADM products in aquaculture.

Publications/Presentations

- Trushenski, J.T., and C.C. Kohler. Vitamin E requirement of sunshine bass as met by the natural source, d-alpha tocopheryl acetate. *North American Journal of Aquaculture* 68:186-191.
- Trushenski, J.T., C.S. Kasper, and C.C. Kohler. 2006. Challenges and opportunities in finfish nutrition. *North American Journal of Aquaculture* 68:122-140.
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- Trushenski, J.T., and C.C. Kohler. 2006. Physiological influence of natural source vitamin E: alterations in metabolism and endocrinology, Invited. *Aquaculture America*, Las Vegas, NV.
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- Trushenski, J.T., and C.C. Kohler. 2004. Nutraceuticals in Aquaculture: Using Vitamin E to Promote Fish Health, Invited. Annual Meeting of the American Fisheries Society, Madison, WI.

Undergraduates/Graduates

The only student, graduate or otherwise, supported by this project is the Industry Fellow, Jesse Trushenski. The project represents Ms. Trushenski's doctoral research which she plans to defend in Fall 2006.

Related Projects

Not applicable.

Patents/Licenses

Not applicable.

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Abstract

Our purpose was to investigate the use of d-Alpha® Natural Source Vitamin E (d-alpha tocopheryl acetate) in prepared diets for sunshine bass to enhance immunity and reduce stress-related mortality. Sunshine bass are well-suited to commercial culture, however, the industry is limited by the immunosuppressive consequences of stress. Administered at higher dosages, the properties of d-Alpha® may stimulate the immune system and compensate for the negative effects of stress. To fully investigate the potential of d-Alpha® as a nutraceutical supplement for sunshine bass, five objectives were developed: 1) determine the vitamin E requirement of sunshine bass as met by d-Alpha®, 2) evaluate the effects of nutraceutical-level supplementation with d-Alpha® on stress tolerance and nonspecific immunity of stressed sunshine bass, 3) evaluate nutraceutical effects of d-Alpha® supplementation in a disease challenge, 4) evaluate the effect of d-Alpha® supplementation on tissue fatty acid (FA) profiles and lipid metabolism during exertion and stress, and 5) conduct cost-effectiveness analyses of d-Alpha® as a nutraceutical feed supplement aquaculture feeds.

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1. Determine the nutritional requirement of sunshine bass for d-Alpha®, using production performance- and non-performance-based indicators of hypo- and hypervitaminosis.
2. Examine the effects of nutraceutical-level supplementation with d-Alpha® on stress tolerance and nonspecific immunity of stressed sunshine bass, and identify a dietary concentration of d-Alpha® that optimizes those parameters.
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4. Examine the effects of nutraceutical-level d-Alpha® supplementation on tissue fatty acid profiles and lipid metabolism during exertion.
5. Conduct cost-effectiveness analyses of d-Alpha® nutraceutical application and using d-Alpha® in prepared feeds for sunshine bass.

Narrative Report, Year 1.

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Following the completion of Objective 1, a second feeding trial was initiated in January of 2005 to begin Objective 2. At the end of the feeding trial, experimental stressors were applied as delineated in the original proposal and all data for Objective 2 were successfully collected. We observed no immediate immunosuppressive effects of stress, however, stressor exposure resulted in increased vulnerability of fillet tissue to oxidative degradation. Increasing dietary NSVE was associated with increased resistance to oxidation of fillet tissue during storage and increased performance in humoral immunocompetence, suggesting NSVE possesses some ability to modulate performance in these response categories. Fillet incorporation of tocopherols was dramatically higher among fish fed increased levels of NSVE, and diet-to-fillet transfer rates observed were substantially greater than those reported for SYNE in other species. Maximal modulatory response was associated with a dietary concentration representing approximately 5 times (5X) the previously determined requirement. Preliminary results were presented to the ADM Task Force in May 2005 at the meeting previously mentioned. Final results were presented to the ADM Task Force in October 2005, at the 2005 Annual Meeting of the American Fisheries Society, Aquaculture America 2006, and AQUA 2006. A manuscript has been prepared and will be submitted for review, likely to Aquaculture Nutrition, in the near future.

Narrative Report, Year 2.

Objective 3 was completed in April of 2006. Diets containing requisite or 5X levels of SYNE or NSVE were compared in a stress and disease challenge (*Flavobacterium columnare*) experiment. Although stimulatory effects were not observed under normal culture conditions, super-requirement supplementation with NSVE compensated for stress and disease challenge-related reductions in immunocompetency of sunshine bass. Stressor exposure generally resulted in depressed immunocompetency, however, the magnitude of immunosuppression was attenuated in some respects among fish fed the NSVE-based diets, particularly those supplemented at super-requirement levels. The overall effect of stress on pathogen exclusion and/or clearance of sunshine bass was evidenced by greater systemic pathogen burden associated with stress-exposed groups. Cumulative dietary impact on resistance of sunshine bass to *F. columnare* is somewhat equivocal. Blood densities of *F. columnare* were lower among SYNE-fed fish within pathogen-exposed, non-stressed groups. When challenged with stress and pathogen exposure, 1X and 5X NSVE groups carried lower *F. columnare* loads than the 1X SYNE group, but bacterial densities were lowest among the 5X SYNE group. This would suggest greater resistance or pathogen clearance among fish fed elevated levels of SYNE, however, this was not clearly supported by the clinical measures of immunocompetency employed in the present work. We concluded the immunomodulatory influence of NSVE is unique and superior relative to SYNE with respect to nonspecific mechanisms of disease resistance. Further, super-requirement dietary levels of NSVE may enhance immunocompetency of sunshine bass during infection and aid in pathogen clearance. These results will be presented during the next ADM Task Force meeting and at the upcoming AQUA 2007 conference. A manuscript based on this project is currently in development.

Objective 4 was completed in August of 2005. Forced exertion was observed to down-regulate adipose phospholipase A₂ (cPLA₂) activity and affect resultant prostaglandin concentrations cPLA₂ activity and prostaglandin concentrations, although these effects were attenuated by an opposing effect of increasing NSVE. Super-requirement levels of d-Alpha® were also associated with increased energy mobilization during exertion of sunshine bass, potentially via optimization of adipose phospholipase activity and/or alterations in prostaglandin concentrations. Lipid metabolism was not significantly affected in this trial, however, increasing d-Alpha® was associated with beneficial shifts in fillet FA composition. Preliminary results were presented to the ADM Task Force in October 2005; final results were presented at Aquaculture America 2006 and AQUA 2006. A manuscript based on this project is currently in development.

Lack of direct, cause-effect relationships between dietary d-Alpha® content and measures of mortality/recovery due to disease precluded exhaustive cost-effectiveness analysis for Objective 5. However, we have demonstrated that d-Alpha® is more biologically potent than SYNE in meeting minimum dietary requirements of sunshine bass. Moreover, when provided at super-requirement levels, d-Alpha® consistently confers benefit beyond that achieved with SYNE. Currently, d-Alpha® is marketed at equivalent cost per unit vitamin E activity to SYNE. Given the increased potency and unique characteristics of d-Alpha®, nutraceutical application of this product may result in decreased cost of production (e.g., reduced feed cost, occurrence of disease) and increased product value (e.g., enhanced fillet tocopherol content, improved fillet stability) in sunshine bass aquaculture.

Potential Applications or Benefits

Natural source vitamin E has not been widely researched in animal nutrition, and the present work is the first to directly evaluate the relative nutraceutical potential of NSVE in an aquaculture species. We have conducted numerous trials with d-Alpha®, and have established a record of rigorous scientific assessment of this product. In addition to the scientific advancement our results represent, our research has added to the field of nutraceutical research in aquaculture. The project has generated a substantial amount of relevant data for Archer Daniels Midland Company (ADM, industry partner and producer of d-Alpha®) as well as garnered national and international interest among academic and industry representatives. We have successfully described the beneficial attributes of d-Alpha®, and have laid the foundation for implementation of this innovative product as a nutraceutical in aquafeeds.

Lay Summary

Stress results in a higher incidence of disease among livestock. Although stress cannot be removed from livestock production, its effects can be reduced through stimulation of the immune system. One means of increasing disease resistance is through the use of nutraceuticals, or dietary components that enhance immune function. These compounds are common in human nutrition (e.g. echinacea, ginseng, ginkgo, various antioxidants, etc.), and are beginning to be investigated in animal nutrition as well. Nutraceuticals are particularly appropriate for the aquaculture because contact with

livestock is essentially limited to feeding, and stress-related disease and losses remains a significant problem for this agricultural industry.

We investigated natural source vitamin E as a nutraceutical for use in sunshine bass (male striped bass crossed with female white bass) feeds. Although vitamin E is an essential nutrient required by all animals, at higher levels above the nutritional requirement, it has been shown to stimulate the immune system of animals, including fish. Natural source vitamin E is a specific chemical form isolated from oilseeds such as soybeans which has a greater potency than the synthetic form typically used in animal feeds. We hypothesized natural source vitamin E would have greater nutraceutical potential due to its greater biological activity. We found the nutritional vitamin E requirement of sunshine bass can be met by lower levels of natural source vitamin E compared to the synthetic form. Subsequent studies based on this initial finding indicated increasing the dietary concentration of natural source vitamin E maintained, or in some cases increased, disease resistance in sunshine bass. Additionally, use of natural source vitamin E may enhance the quality of aquaculture products by increasing fillet vitamin E content and shelf-life. In short, use of natural source vitamin E, particularly at higher dietary concentrations, is beneficial to aquatic livestock and can enhance product value.

International Implications

Sunshine bass are a primary U.S. aquaculture species, however our results may be applicable to other aquaculture species raised throughout the world. Along these lines, an Australian research team has expressed interest in developing similar applications of d-Alpha® in tuna aquaculture, and Ms. Trushenski is currently in contact with this team to develop potential applications. ADM, a multi-national company, will benefit from the scientific information provided by the project and will likely utilize the information we have provided in application and sales of d-Alpha®.

Media Coverage

In addition to the online announcements/articles published by Illinois-Indiana and National Sea Grant Offices, the fellowship program and this project has been featured in "Perspectives", a publication of Southern Illinois University Carbondale covering excellence in research (hard copy included). An article also appeared on the SIUC news website (hard copy included). The project was also featured multiple times on broadcasts of SIUC radio.

Partnerships

The project has continued reinforce the research collaboration between ADM and the Fisheries and Illinois Aquaculture Center. The specific outcomes of this project, as well as the collaborative environment it fosters, have led to several additional projects currently in development to evaluate application of other ADM products in aquaculture.

Publications/Presentations

- Trushenski, J.T., and C.C. Kohler. Vitamin E requirement of sunshine bass as met by the natural source, d-alpha tocopheryl acetate. *North American Journal of Aquaculture* 68:186-191.
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- Trushenski, J.T., and C.C. Kohler. 2006. Physiological influence of dietary natural source vitamin e during homeostatic disturbance: alterations in metabolism, endocrinology, and immunity of sunshine bass *Morone chrysops* x *M. saxatilis*. AQUA 2006, Florence, Italy.
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Undergraduates/Graduates

The only student, graduate or otherwise, supported by this project is the Industry Fellow, Jesse Trushenski. The project represents Ms. Trushenski's doctoral research which she plans to defend in Fall 2006.

Related Projects

Not applicable.

Patents/Licenses

Not applicable.

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