第２０回生物資源環境科学府賞
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資源生物科学専攻　　家畜生体機構学分野
「Studies on the elucidation of central functions of taurine to regulate body temperature, food intake and stress response in neonatal chicks」

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環境農学専攻　　農産食料流通工学分野
「Development of chitosan-based nanocomposite enriched with essential oil as antifungal coating for fresh fruit application」

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Title　：Studies on the elucidation of central functions of taurine to regulate body temperature, food intake and stress response in neonatal chicks
(ニワトリヒナの体温、摂食およびストレス応答を制御するタウリンの中枢機能の解明に関する研究)

Thesis Abstract

Environmental stressors in poultry such as heat stress, social isolation stress, cold stress, etc. negatively affect poultry performance, health and welfare. These stressors cause physiological alterations, behavioral changes, reduction in production and high mortality. Amino acids are getting attention nowadays as a novel approach to mitigate stress responses in poultry. In this study, a novel role and functional mechanism of taurine have been investigated to mitigate stress response in neonatal chicks.

My research finding revealed that central taurine induced dose-dependent hypothermia and reduced food intake in neonatal chicks under control thermoneutral temperature. Further, it was found that γ-aminobutyric acid $_A$ ($GABA_A$) receptor mediated taurine-induced hypothermia. In addition, central taurine afforded thermotolerance with rapid initiation of heat dissipation behaviors; namely, panting and wing dropping, compared with control birds along with the modification of mitochondrial thermogenic gene expressions. Furthermore, central taurine attenuated hyperthermia and isolation stress behaviors in chicks through inducing sedative and hypnotic effects. Moreover, norepinephrine and serotonin were found to mediate taurine-induced hypothermia; however, serotonin alone may be linked with taurine-induced anorexia. Furthermore, changes in several amino acid concentrations by central injection of taurine further suggest that amino acid metabolism may change to contribute to the central thermoregulatory and stress attenuating functions of taurine.

In conclusion, the present study revealed a novel nutritional factor, taurine to alleviate heat stress and isolation-stress response in chicks. Several mechanisms have been investigated to reveal the regulatory functions of taurine in the brain including the mechanisms of $GABA_A$ receptor, amino acids and monoamines. Furthermore, the central regulatory mechanisms of taurine provided a better understanding of thermoregulation, appetite regulation and stress response, which has far-reaching implications in a multitude of species and biological contexts.

Development of chitosan-based nanocomposite enriched with essential oil as antifungal coating for fresh fruit application

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SUMMARY

Edible packaging (coatings and films) has been used to improve the shelf life of fresh fruit produce. The preparation process for edible packaging depends on the properties of the ingredient and the application end use. The objective of this study were to 1) develop antifungal nanocomposite coating formulated from chitosan (Chi), Indonesia-originated essential oil, and nanoparticles; 2) characterize the functional properties of the coating film. This was done by optimizing the raw material dosage selection, and blending sequence.

In the first work, the properties of an antifungal coating film made from Chi combined with zinc oxide nanoparticles (ZNPs) and Indonesian sandalwood essential oil (SEO) were investigated. Incorporation of 0.5% SEO or 0.025% ZNPs plus 0.5% SEO into 0.8% Chi coating solution showed outstanding effects on the inhibition of both mycelium growth and spore germination of *Penicillium italicum*. The antifungal mechanism was explained by measuring the loss of membrane integrity of spores. The antifungal effectiveness was confirmed in vivo by carrying out tests on tangerine fruit. The biocompatibility of these coatings was

analyzed using Fourier transform infrared spectroscopy, scanning electron microscopy, atomic force microscopy, and fluorescence microscopy. Significant increases in pH, appearance viscosity, and transparency and a decrease in light transmittance were found with 0.8% Chi plus 0.025% ZNPs and 0.5% SEO compared with the control. Chi films incorporating SEO and ZNPs can be used as an edible film and coating to reduce chemical use.

Further work, a novel formulation of composite coating comprising 0.8% Chi incorporating 0.025% copper(II) oxide nanoparticles (CuO) and 0.5% Indonesian cedarwood essential oil (CEO) was fabricated by casting method. FTIR, CLSM, and SEM analyses were employed to characterize the biocompatibility of each formulation. Additionally, the physico-chemically properties of the composite coatings were characterized. The colour (L^*), light transmission, zeta potential, and roughness of Chi were significantly ($P < 0.05$) altered negatively by the presence of CuO or CEO; the colour (a^*, b^*, and ΔE), apparent viscosity, and transparency also changed positively as a consequence of CuO and CEO incorporation. The antifungal features of a pure Chi coating against *Penicillium italicum* and *Penicillium digitatum* were improved synergistically by CuO and CEO, confirmed by *in vitro* and *in vivo* assays. Composite coatings obtained in this work may have potential applications for active primary food packaging, particularly for fresh postharvest commodities.

Lastly, a novel composite edible coating film was developed from 0.8% Chi and 0.5% sandalwood oil (SEO). Cellulose nanofibers (CNFs) were used as a stabilizer agent of oil-in-water Pickering emulsion. We found four typical groups of CNF level-dependent emulsion stabilization, including (1) unstable emulsion in the absence of CNFs; (2) unstable emulsion (0.006–0.21% CNFs); (3) stable emulsion (0.24–0.31% CNFs); and (4) regular emulsion with the addition of surfactant. Confocal laser scanning microscopy was performed to reveal the characteristics of droplet diameter and morphology. Antifungal tests against *Botrytis cinerea*

and *Penicillium digitatum*, between emulsion coating stabilized with CNFs (Chi/SEO $_{Pickering}$) and Chi or Chi/SEO was tested. The effective concentration of CNFs (0.24%) may improve the performance of Chi coating and maintain Chi/SEO antifungal activity synergistically confirmed with a series of assays (*in vitro*, *in vivo*, and membrane integrity changes). The incorporation of CNFs contributed to improve the functional properties of Chi and SEO-loaded Chi including light transmission at UV and visible light wavelengths and tensile strength. AFM and SEM were employed to characterize the biocompatibility of each coating film formulation. Emulsion-CNF stabilized coating may have potential applications for active coating for fresh fruit commodities.

In summary, this doctoral dissertation proposed new functional coating materials as an alternative to plastic packaging by developing chitosan-based agents that were effective in preserving the quality of fresh fruits and preventing spoilage through its antimicrobial effect.