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# Validation of a luciferase bioassay to detect the progestative activity in gilts whose estrus was induced by an uterotonic herb (*Ligusticum chuanxiong*)

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ABSTRACT

*Ligusticum chuanxiong* (LC) is an uterotonic herb. Ethanol extract of LC has potent progestative activity. The progestative activity in rat serum was well documented after oral and subcutaneous administration of LC. The objective of this study was to investigate the possibility of LC for estrus synchronization of gilts in place of synthetic progesterone. Eighteen gilts were randomly assigned into three groups, control group fed normal feed, positive control group supplemented with 20 mg of altrenogest (Regumate®) and treatment group supplemented with 100 g of LC daily for 22 consecutive days in feed. Blood samples were collected from jugular vein of gilts on the 22nd day before altrenogest or LC feeding. Estrus was monitored every day in the morning and the evening by observing the vulva swelling and reddening as well as nose to nose fence line contact between the boar and sow. Estrus was confirmed by testing the standing reflex in the presence of boar. All gilts in LC-fed group and 5 gilts in altrenogest-fed group came in estrus within 2–6 days after withdrawal of feeding, but did not show estrus during the feeding period. Whereas, all gilts in control group came in estrus during the feeding period. Progesterone responsive reporter plasmid was constructed by incorporating the progesterone response elements and TATA box at the multiple cloning site of pGL3 basic vector. Gilts serum progestative activities were measured by using luciferase reporter gene bioassay. Serum analysis showed that progestative activities of altrenogest- and LC-supplemented groups had a similar pattern. It is concluded that LC is a potential feed additive to be used for estrus cycle synchronization in swine industry.



## 1. Introduction

The normal estrus cycle in sow is 21 days long (Senger, 2003) and it starts from 5–7 months of age (Brinkley, 1981). Estrus synchronization involves interrupting the natural estrus cycles of female animals so they can be pregnant at approximately the same time. Balanced and feedback mechanism of gonadotropins releasing hormone

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(GnRH), follicle stimulating hormone (FSH), luteinizing hormone (LH), estrogen and progesterone are important for ovarian activities such as follicular development, maturation as well as ovulation (Lesoon and Mahesh, 1992). FSH and LH are low at the prepubertal stage in gilts (Parlow et al., 1964). However, prior to the first estrus, anterior lobe of pituitary gland secretes higher level of FSH and LH under the stimulation of GnRH for follicle development and ovulation (Driancourt et al., 1995). On the other hand, progesterone inhibits the synthesis and secretion of GnRH which subsequently inhibits the follicular growth and development as well as ovulation (Lesoon and Mahesh, 1992). Protocols have been developed for estrus synchronization of gilts, either by controlling events leading to follicles development, maturation and ovulation or altering luteal phases. Progesterone and its derivatives were not proved fully effective for estrus synchronization of gilts (First et al., 1963). For this reason, non-steroidal progestogen, such as methallibure (Aimax®, ICI33828, Suisynchron®), allyl-trenbolone (altrenogest, as Regumate®) as well as other hormones including PG600® (combination of equine chorionic gonadotropin and human chorionic gonadotropin), Prostamate® and Lutalyse® ($PGF_{2\alpha}$) are commercially used in swine industry (Britt et al., 1989, Estienne et al., 2001; Kaeoket, 2008). Due to the teratogenic effect of methallibure and Zinc-methallibure, they were banned by US and EU in swine industry. Nowadays, allyl-trenbolone is the only substance with progestative effect used for swine estrus synchronization around the world. Although allyl-trenbolone is highly effective in mature and random cycling gilts, it is not effective for estrus induction in prepubertal gilts. Therefore, it is necessary to find new substances with progestogenic effect.

*Ligusticum chuanxiong* Hort. (family Apiaceae) is known as uterotonic herb and commonly used in traditional Asian folk medicines. The strong progestative activity of its ethanolic extract was well documented. The maximal progestative activity of the LC ethanolic extract was comparable to 95% of 100 nM progesterone with an $EC_{50}$ of 7 μg/ml (Lim et al., 2006a). Oil fraction of LC extract contains monomeric and dimeric Phthalides and it is prescribed for several diseases including menstrual disorders (Chen, 1992; Zhang et al., 2003). Two phytoprogesterone, 3,8-dihydro-diligustilide and Riligustilide, were isolated from LC extract, and 3,8-dihydro-diligustilide has higher progestative activity. The maximal progestative activity of 3,8-dihydro-diligustilide was equivalent to 180% of 100 nM progesterone with an $EC_{50}$ of 91 nM (Lim et al., 2006b). Riligustilide was less strong than 3,8-dihydro-diligustilide and its maximal activities was 15% of 100 nM progesterone with an $EC_{50}$ of 81 μM (Lim et al., 2006a). Phyto-progestagens in LC were efficiently absorbed and the progestogenic activity was detected in rat sera when administrated subcutaneously and orally (Lim et al., 2006b). Phyto-progestagens in LC crude extract act through the same molecular mechanisms of progesterone agonist (Lim et al., 2006a). The progestative activities of LC extracts were fully suppressed by progesterone antagonist, RU486 (Lim et al., 2006b). Therefore, LC could potentially be used as an alternative for progesterone. The objectives of the study were to investigate the possibility of using LC for estrus synchronization of gilts and to develop and validate a bioassay to detect serum progestative activity of LC extract.

## 2. Material and methods

### 2.1. DNA vectors, chemicals and reagents

The pGL3 basic and pSV-β-galactosidase control vector, luciferase enzyme assay system, β-galactosidase enzyme assay system and restriction enzymes were purchased from Promega (Madison, WI, USA). Progesterone, estradiol-17β, testosterone, dexamethasone and mifepristone (RU486) were bought from Sigma Aldrich (St. Louis, MO, USA). Lipofectamine 2000, Dulbecco's Modified Eagle Medium (DMEM) and fetal bovine serums (FBS) were obtained from Invitrogen (Carlsbad, CA, USA).

### 2.2. Design of animal experiment

Eighteen crossbred (Landrace − Duroc × Yorkshire) gilts with an average age of 6–8 months and average bodyweight of 120 kg were used. When observing the first estrus in pig farm, gilts were randomly divided into three groups with 6 animals each, control group fed only normal commercial feed, positive control group supplemented with 20 mg altrenogest once daily in the morning and treated group supplemented with 100 g LC powder twice daily in the morning and the evening, in the feed for 22 consecutive days. All gilts were fed a commercial feed at 2 kg/gilt in individual trough twice a day at 8 AM and 5 PM. Water was available *ad libitum*. Estrus detection was performed twice every day, starting on the first day of feeding until 7 days after withdraw of altrenogest and LC. Estrus was recognized by three healthy 10–12 months aged boars which were housed other end of the breeding barn from the gilts. Gilts were exposed to the boar two times daily, in the morning and the evening for 20–30 min each time, boars were allowed to move through the alley of each gilt pen, nose to nose fence line contact between the boar and each sow was observed, and then back pressure act by hand was practiced to confirm the immobility of gilts. In addition, red and swollen vulvas were also monitored every day in the morning and the evening. Before feeding on the last day of experiment, 6 ml of blood sample was collected from jugular vein of each gilt, allowed for clotting on ice and then centrifuged at 1500*g* for 30 min at 4 °C and kept at −20 °C.

### 2.3. Preparation of LC extracts

Rhizome chuanxiong (*L. chuanxiong*) roots, purchased from Sun Ten Pharmaceutical Co. Ltd. (Taichung, Taiwan), were grinded and socked with 100% ethanol for 72 h at RT. The extracts were filtrated and kept inside a hood for rapid evaporation of ethanol. Dried extracts were diluted at the 100 mg/ml stock concentration and kept at −80 °C.