# EFFECTS OF DAIDZEIN FEEDING OF PREGNANT SOWS ON CARCASS AND MEAT QUALITY AND SKELETAL MUSCLE CELLULARITY OF THE PROGENY

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## Introduction

Biologically active compounds in the maternal circulatory system may influence not only the maternal organism but also the foetus. In this context dietary phytoestrogens are of interest, as they are able to cross the placenta. Phytoestrogens are plant-derived compounds with structural and functional similarities to natural estrogens (Ren *et al.*, 2001a). Soy and soy products are the most significant dietary sources of isoflavonic phytoestrogens, mainly of genistein and daidzein (Barnes *et al.*, 1994). In pigs, the level of circulating isoflavones and thus their bioavailability depends on the isoflavone content of the soy-product in the diet (Kuhn *et al.*, 2004). Isoflavones have been shown to exhibit multi-biological properties including estrogenic and anti-estrogenic effects, inhibitory effects on protein tyrosine kinases (e.g. growth factor receptors), modulation of gene transcription, and antioxidant actions (Ren *et al.*, 2001a). From these various features both promoting and inhibiting properties of isoflavones on cellular growth and development are to be expected. Results on the influence of isoflavones on growth and differentiation of porcine skeletal muscle are not yet available. To better understand the significance of isoflavones contained in feed on pig performance, the aim of this study was to investigate the effects of maternal dietary daidzein supplementation during late gestation on the progeny at birth and at market weight with focus on body composition, carcass and meat quality, and associated skeletal muscle cellularity.

## Materials and Methods

*Animals and feeding.* Sixteen multiparous sows (German Landrace) were bred to the same German Landrace boar and housed individually. All sows were fed twice daily with a special soybean-free triticale-wheat based diet (2.6 up to 5 kg/daily) containing 13.0 MJ metabolizable energy per kg dry matter, 16% crude protein, and 0.94% lysine. Sows were divided into experimental (n=8) and control (n=8) groups with balanced live weights and parity. From day 85 of gestation until parturition the diet of the experimental group was supplemented with 1 mg of pure daidzein (Nanjing Agricultural University, China) per kg body weight and day. This approximates a concentration of daidzein (c50 mg/kg feed) as it has been measured in a common triticale-soybean meal diet (Kuhn *et al.*, 2004a). After birth two male piglets per litter were collected for detailed analysis. The remaining piglets were reared until market weight and slaughtered at 180.4±5.3 days of age (n=66 control; n=55 daidzein).

*Piglet body composition, carcass and meat quality* were analyzed as described previously (Kuhn *et al.*, 2004b). *Muscle histology and biochemistry.* Samples from *semitendinosus* (ST) muscle were collected from newborn piglets and slaughter pigs. Neonatal muscle was further analyzed according to Rehfeldt *et al.* (2001). In adult pigs, muscle fibre characteristics were measured in a sample of 29 controls and 25 experimental (daidzein) pigs only. Samples from the ST midbelly were taken, mounted on cork-chucks and snap-frozen in liquid nitrogen. Serial sections of 10 µm were stained for cytoplasm/nuclei by haematoxylin/eosin or exposed to a combined reaction for NADH-TR and ATPase at pH 4.2, which enables to classify muscle fibre types (STO, FTO, FTG) (Rehfeldt *et al.*, 1993). Microscopic evaluation was carried out by image analysis (AMBA, IBSB, Berlin, Germany). Homogenates of neonatal ST muscle were used to quantify DNA, protein, and creatine kinase (CK) activity as described previously (Rehfeldt *et al.*, 2001); RNA was quantified with SYBR®Green II (Oksbjerg *et al.*, 2000). *Statistical analyses.* Data were subjected to ANOVA by the GLM or mixed classification models of SAS (SAS Inst. Inc., Cary NC). Significance of LSmeans differences was tested by the Student's t-test ($P<0.05$).

## Results and Discussion

*Effects on newborn piglets.* Maternal performance, such as litter size, percentages of live born piglets and runts, average birth weight per litter and litter weight, was not different between control and daidzein-supplemented sows (data not shown). In contrast, Ren *et al.* (2001b) found increased birth weights in males in response to maternal daidzein, but at a very low doses supplemented to a normal soy-based diet. In our study, maternal daidzein elevated the concentration of fat in the whole piglet body suggesting that overall development was positively influenced (Kuhn *et al.*, 2002) without being apparent as higher birth weight. Average tissue proportions of the piglets were not changed in response to daidzein feeding (Table 1). Likewise, neither muscle weight nor histological and biochemical properties were significantly affected, even though numerical decreases in fibre number, protein and CK activity were seen. However, the response to maternal daidzein depended on litter size as seen by significant interactions. The percentage of muscle tissue tended to decrease by maternal daidzein feeding in piglets belonging to large litters ($P$=0.09), whereas the percentage of skin increased ($P$=0.03). Accordingly, muscular CK activity was lower ($P$=0.06) suggesting that myogenesis was slightly impaired by daidzein in piglets that highly compete for maternal nutrients. Recent results of an *in vitro* study revealed that daidzein and genistein inhibit the growth of porcine muscle satellite cells in a dose-dependent manner (Mau *et al.*, 2006). Litter size (≥15 vs. <15), but not sex, was an influential factor for birth weight and maturity as seen by lower body fat percentage, ST muscle weight, myofibre numbers, and CK activity in piglets from large litters.

*Effects on postnatal growth, carcass and meat quality.* Postnatal growth, carcass and meat quality at market weight were not changed by maternal daidzein feeding (not shown). Likewise, average meat quality measured on the *longissimus* muscle of the progeny, was not influenced. However, the pigs originating from large litters exhibited higher muscle $pH_{45}$ (P=0.02) indicating a slight improvement of meat quality by maternal daidzein, but the mechanism remains unclear. Carcass weight and composition were sex-dependent. Male castrates showed greater live and carcass weights, back fat thickness, but lower meat percentage and loin area (see also Correa *et al.*, 2006), and different meat quality and muscle structure than females.

**Table 1:** Body composition and *semitendinosus* (ST) muscle characteristics of male neonatal piglets (LSmeans ± SE) born to sows supplemented with dietary daidzein of 1 mg/kg body weight/d from d 85 of gestation to parturition as compared to untreated controls.

| Item | Control | Daidzein | *P* : T[a] | L[b] | T x L |
|---|---|---|---|---|---|
| Birth weight, g | 1,266 ± 50 | 1,272 ± 46 | 0.93 | 0.01 | 0.53 |
| Meat, % | 43.4 ± 0.60 | 42.9 ± 0.66 | 0.64 | 0.68 | 0.07[e] |
| Subcutaneous fat, % | 8.6 ± 0.35 | 9.0 ± 0.39 | 0.50 | 0.32 | 0.79 |
| Bones, % | 36.6 ± 0.75 | 36.4 ± 0.83 | 0.84 | 0.56 | 0.20 |
| Skin, % | 10.6 ± 0.16 | 11.0 ± 0.18 | 0.14 | 0.26 | 0.10[f] |
| Protein, % | 14.75 ± 0.24 | 15.76 ± 0.27 | 0.99 | 0.63 | 0.96 |
| Fat, % | 1.19 ± 0.04 | 1.30 ± 0.04 | 0.04 | 0.01 | 0.85 |
| ST Muscle weight (g) | 2.77 ± 0.20 | 2.63 ± 0.22 | 0.65 | 0.05 | 0.28 |
| ST Protein (mg/g) | 91.2 ± 4.3 | 86.7 ± 4.7 | 0.50 | 0.14 | 0.79 |
| ST CK (IU/mg protein)[c] | 3.76 ± 0.28 | 3.58 ± 0.22 | 0.56 | 0.06[d] | 0.76 |
| ST Total fibre number | 458.3 ± 29.6 | 409.4 ± 32.4 | 0.30 | 0.03 | 0.51 |

[a]T – treatment; [b]L – litter size group; [c]CK – creatine kinase activity; [d]lower in large litters (*P*=0.06); [e]Decreases in large litters (*P* = 0.09); [f]Increases in large litters (*P* = 0.03)

**Conclusions**

The isoflavone compound daidzein when supplemented to a soy-free sow diet during late gestation in an amount typical for soy-based diets marginally affects growth, carcass and meat quality, and skeletal muscle cellularity of the progeny. Slight detrimental effects on foetal muscle development in piglets from large litters can be compensated for during postnatal growth. Higher doses and/or other kinds of isoflavones might have greater effects on muscle growth and meat quality.

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