140 **The influence of increasing dietary methionine on the performance of the early weaned pig (10 ± 4 d of age).** K.Q. Owen*, J.L. Nelssen, R.D. Goodband, M.D. Tokach, S.S. Dritz, L.J. Kats, and B.T. Richert. Kansas State University, Manhattan.

Four hundred thirty-five pigs (initially 3.5 kg and 10.1 ± 4 d of age) were used on a commercial farm to determine the influence of increasing dietary methionine on growth performance for the early weaned pig (10 d of age). Pigs were blocked by weight in a randomized complete block design resulting in six to thirteen pigs per pen and a total of eight pens per treatment. Experimental diets were fed from d 0 to 21 postweaning. Dietary methionine levels were achieved by adding increasing liquid methionine (Alimet) to a common basal diet. The control diet was corn based and contained 8.7% moist extruded soy protein concentrate, 10% spray-dried porcine plasma, 25% dried whey, 5% dried skim milk, 3% fish meal, and 1.75% spray-dried blood meal. All diets were formulated to contain 1.8% lysine. Liquid methionine replaced sucrose in the control diet to provide dietary methionine levels of .36, .40, .44, .48, .52, and .56%. Each diet contained .62% cystine and 704 g of added choline chloride (60%). Blood samples were collected on d 7 and 14 to determine plasma urea N (PUN). During d 0 to 7 postweaning, ADG and feed efficiency (G/F) were improved (linearly, $P < .01$) with increasing dietary methionine with optimal performance observed between .48 and .52% dietary methionine. However, ADFI was not effected by dietary methionine. For the entire period (d 0 to 21 postweaning), ADG and G/F were improved (linear, $P < .05$) with increasing dietary methionine and maximized between .48 to .52% dietary methionine. On d 7 postweaning, plasma urea nitrogen was reduced (quadratic, $P < .10$) as dietary methionine increased with pigs fed the .52% methionine having the lowest plasma urea nitrogen concentrations. These data suggest that the early weaned pig (d 10) needs approximately .48 to .52% dietary methionine when fed a diet containing 1.8% lysine to optimize growth performance. This establishes that a methionine to lysine ratio of approximately 28% exist for the 10 d old pig, similar to that for the 6 to 20 kg pig. This research confirms earlier results from our laboratory and the University of Illinois indicating that the optimal methionine to lysine ratio is approximately 28% for pigs from 3.5 to 20 kg BW.

		Dietary Methionine, %						
Item		.36	.40	.44	.48	.52	.56	CV
d 0 - 7	ADG, g[a]	105	117	128	133	140	141	22.9
	ADFI, g	123	124	134	127	136	134	14.5
	G/F[a]	.85	.95	.95	1.03	1.00	1.03	13.6
d 7	PUN, mg/dL[b]	13.2	13.3	12.4	11.9	11.4	13.3	12.0

[a] Linear effect of dietary methionine ($P < .01$).
[b] Quadratic effect of dietary methionine ($P < .10$).

Key Words: Methionine, Pigs, Growth performance.

PHYSIOLOGY

141 **Effects of extracellular potassium on the meiotic and cytoplasmic maturation of porcine oocytes.** N. H. Kim*, H. Funahashi, T.C. Cantley, T.T. Stumpf and B.N. Day, University of Missouri-Columbia, Columbia, MO

Effects of extracellular potassium concentration in maturation media on the meiotic and cytoplasmic maturation of porcine oocytes were examined. Oocyte-cumulus complexes from slaughterhouse ovaries were cultured in BSA-free Whitten's medium containing 0, 3, 6, 12 and 16 mM potassium supplemented with 0.4% polyvynylalcohol (PVA) or 10% porcine follicular fluid (pFF), 10 iu PMSG/ml, 10 iu hCG/ml and 1 μg estradiol/ml for 20 h and then without these hormonal supplements for an additional 20 h. Potassium concentrations were varied by adjusting KCl and KH_2PO_4 concentration. Osmolarity and Cl^- concentrations were maintained by adjusting NaCl concentration. After culture for maturation, oocytes were co-cultured with spermatozoa in modified Medium 199 with 5 mM caffeine and 0.4% BSA for 6 h. Subsequent to IVF oocytes were cultured in Whitten's medium containing 1.5% BSA for 6 h. At this point sperm penetration and male pronuclear formation of the oocytes were determined. Temporal progression of meiotic maturation of porcine oocytes cultured in a medium with 0 (K^+ free) or 6 mM potassium supplemented with 0.4% PVA was also determined after 12, 24, 36, 48 and 60 h of culture. There were no differences in germinal vesicle breakdown (GVBD) and meiotic maturation among 0, 3, 6, 12 and 16 mM potassium containing pFF after 48 hour culture. After 36, 48 and 60 hour of culture, the percentage with GVBD was not different between 0 and 6 mM potassium supplemented with PVA, however further meiotic maturation of oocytes in K^+ free media was inhibited as compared to the 6 mM potassium concentration ($P< 0.01$). The rates of sperm penetration were not different among treatments with pFF ($P> 0.1$), however male pronuclear formation (51.7%, 45/87) in oocytes in medium with 6 mM potassium was higher ($P<0.05$) than those (13.9%, 11/79 and 9.9%, 8/81, respectively) in media with 12 and 16 mM potassium. These results suggest that extracellular potassium is required for meiotic maturation after GVBD, and high concentrations (12 and 16 mM) of potassium in the maturation media impair cytoplasmic maturation.

Key Words: porcine, potassium, oocyte maturation

142 **Effect of TGFβ1 on bovine thecal cell steroidogenesis in vitro** M.J. Al-Hassan* and A.J. Roberts, USDA/ARS MARC, Clay Center, NE 68933

To determine the effect of TGFβ1 in the presence or absence of ovine LH on in vitro steroid secretion by bovine thecal cells, luteal-phase cows (n=5) received a single i.m. injection of prostaglandin $F_{2\alpha}$ (PGF; 25 mg) to induce luteal regression. Cows were randomly assigned to be slaughtered 24 h (n=3) or 48 h (n=2) after PGF, and ovaries were collected immediately after slaughter and transported to the laboratory on ice. Follicles were dissected free of surrounding stroma, and, based on their diameter, were classified as small (≤ 5 mm), medium (5 to 7.9 mm), or large (≥ 8 mm). For large follicles, follicular fluid was aspirated, theca interna layers were microdissected from surrounding stroma, and, based upon follicular fluid estradiol (E2) and progesterone (P4) concentrations, were classified as healthy (E2:P4>1) or atretic (E2:P4<1). For small and medium follicles, follicular fluid and thecal layers were collected and pooled within a cow by size class. Thecal layers were monodispersed enzymatically and cultured (1 x 10^5 cells/well) for 48 h in DMEM/F12 + 0.1% BSA with no treatment (control) or various doses of TGFβ1 (0.25, 25, 2500 pg/ml) with or without ovine LH (500 ng/ml). After culture, conditioned media were collected and stored (-20 °C) until assayed for P4 and androstenedione (A4) concentrations by RIA, and cellular DNA content (ug) was determined for each well. Steroid concentrations are expressed as ng/ug DNA. (Table).

Follicle Type	A4 (ng/ug DNA)	P4 (ng/ug DNA)
Large Healthy	28.68 ± 1.39[a]	.34 ± .31[a]
Large Atretic	3.34 ± 1.39[b]	6.72 ± .40[b]
Medium Pool	10.91 ± 1.20[c]	1.96 ± .29[c]
Small Pool	6.93 ± .93[b]	1.14 ± .21[d]

[abcd] LS Means within a column with different superscripts differ ($P<.05$).

Under the conditions used in this study, TGFβ1 had no effect on secretion of A4 and P4, whereas addition of LH stimulated ($P<.01$) secretion of A4 but not P4 by thecal cells. These results indicate that TGFβ1 has no effect on the steroidogenic activity of cultured bovine thecal cells.

Key Words: TGFβ1, thecal cells, steroidogenesis