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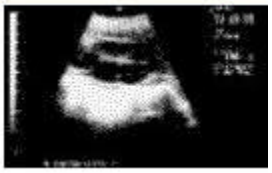


Physiology

CHARACTERIZATION OF COLLAGEN DEGRADATION MARKER EXCRETION IN LACTATING SOWS: A POTENTIAL NON-INVASIVE MEANS TO MONITOR UTERINE INVOLUTION?

B.A. Belstra, W.L. Flowers, M.T. See, and W.J. Croom[1]

Summary

Urinary excretion of the collagen degradation markers deoxypyridinoline and pyridinoline was measured around farrowing, during lactation and postweaning in two separate experiments. Experiment I (n = 5 sows) data suggest that the excretion of both markers increased during early lactation and then decreased prior to weaning but that this pattern is highly variable between sows. Experiment II (n = 21 sows) confirmed the variation between sows and revealed several sows (7 of 21, 33%) that did not exhibit a significant increase in marker excretion. None of the sow or litter variables recorded explained this variability. Comparison of such collagen degradation marker excretion data to a physical measure of uterine involution should reveal how closely these two processes are related and why so much variation between sows exists.

Introduction

Tissues of the postpartum uterus must undergo a degenerative and regenerative process known as involution to prepare a suitable environment to establish the next pregnancy. Based on physical (weight and length) and histological data, uterine involution is complete by about 17 to 21 d postpartum in the sow (see review, Kiracofe, 1980). However, sows that lactate for < 21 d often exhibit reduced embryo survival, conception rate and subsequent litter size (Varley, 1982). This reduced reproductive performance following short lactations is thought to be a consequence of insufficient time to complete involution, although a direct link has not been established (Kiracofe, 1980). Given that average lactation length (weaning age) in US herds has decreased from 28.8 to 18.0 d over the last ten years (PigCHAMP®, Inc., 2000), finding the cause of the reproductive problems associated with early weaning and methods to cope with them may become a critical issue.

During the involution process sow uteri exhibit a 90% degrease in wet weight (from 2.7 to 0.3 kg) as approximately 0.7 to 0.9 kg of collagen is degraded. Small molecules called crosslinks, that link the ends of the fibrils that form a collagen molecule and adjacent collagen molecules together are released as mature collagen is degraded. One of the crosslinks we studied named deoxypyridinoline (Dpd) is found almost exclusively in bone while the other crosslink named pyridinoline (Pyd) is found in many collagenous tissues throughout the body including the uterus (Eyre et al., 1984). Our objective was to characterize the excretion of these two novel collagen degradation markers during lactation. Our ultimate goal is to determine how the excretion of such markers relates to the process of uterine involution and whether or not they can be used as a means to make sow management decisions such as when to wean and which sows might be candidates for pharmacological intervention.

Materials and Methods (Experiment I)

Urine samples were collected from five Yorkshire sows every other day from the day before

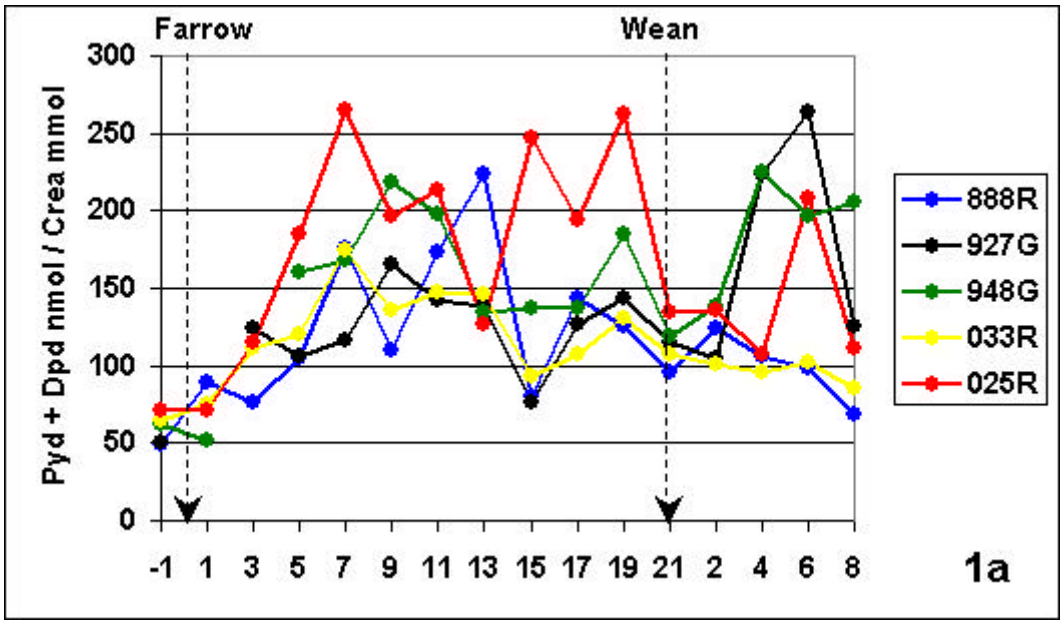
farrowing (–1 d), through lactation (1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21 d) and postweaning (2, 4, 6, 8 d). All five sows farrowed on the same day and lactated for 21 d. Sows were housed in farrowing crates, had ad libitum access to water, and consumed approximately 6.3 to 8.2 kg of a corn-soybean meal based diet daily (15.8% CP, 0.85% lysine and 3307 kcal ME/kg). Data on sow and litter performance were recorded. Every attempt was made to collect urine samples between 0630 and 0830 h but a few samples were not obtained until later in the day during early lactation and postweaning. Urine samples were stored at -20°C and later subjected to a colorimetric assay for creatinine and two enzyme immunoassays (EIA) for markers of collagen degradation (Pyrilinks® and Pyrilinks®-D, Quidel Corp., Santa Clara, CA). The Pyrilinks® assay measures free urinary Pyd and Dpd. The Pyrilinks®-D assay is specific for free urinary Dpd. A sow urine pool was used to establish parallelism and the necessary sample dilution. Intra and inter-assay coefficients of variation were < 6% for all three assays. Pyd and Dpd data are expressed as a ratio to creatinine to standardize variation in urine excretion rate. Pyd and Dpd ratios were graphed and compared to sow and litter variables by computing correlation coefficients (SAS, 1990).

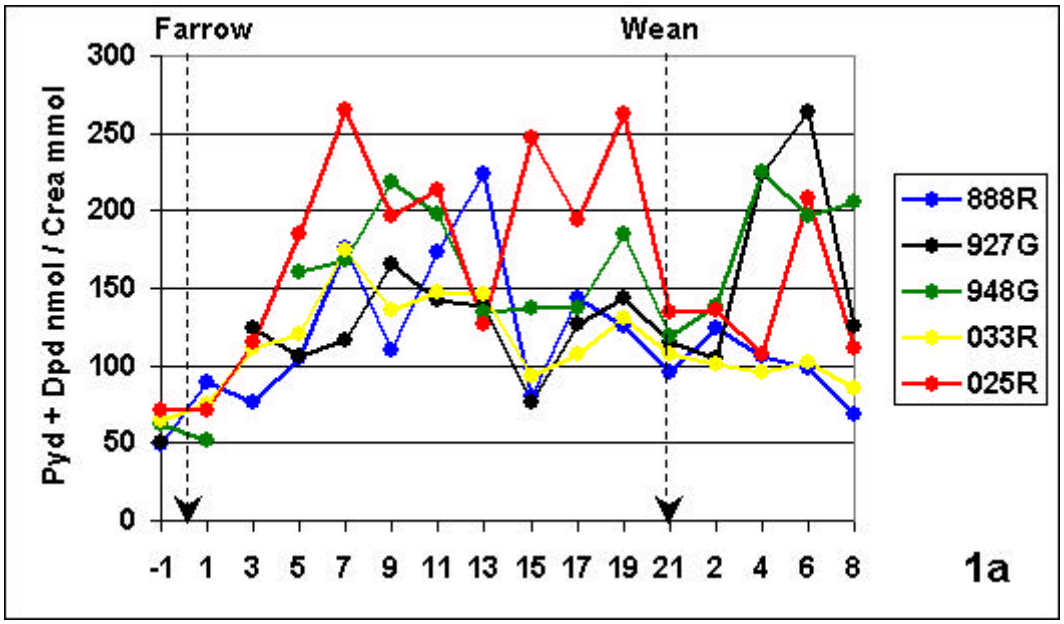
Results and Discussion (Experiment I)

Most of the sow and litter variables (Table 1) were not significantly correlated with the mean or standard deviation of sow Pyd and Dpd ratios. Litter weight weaned was positively correlated ($r = .90$, $P < .04$) with the standard deviation of sow Pyd and Dpd ratio (Figure 1a, Pyrilinks® assay). It seems logical that sows that produced the most milk and litter growth would probably lose the most weight and exhibit the greatest variation in Pyd and Dpd ratio. Total born and born alive were negatively correlated ($r = -.90$, $P < .04$ and $r = -.91$, $P < .04$, respectively) with Dpd ratio (Figure 1b, Pyrilinks-D® assay). This correlation is not logical because Dpd is primarily derived from the degradation of bone collagen and sows that farrowed and suckled the largest litters, not the smallest, should have exhibited the greatest variation in Dpd ratio. It is interesting that the Pyd + Dpd ratio (Figure 1a) at weaning (21 d, 100 to 140) was higher than the pre-farrowing ratio (-1 d, 50 to 75). We had expected the Pyd + Dpd ratio to return to baseline levels (pre-farrowing) prior to weaning. Note the different y-axis scale in figures 1a and 1b. The antibody for the Pyrilinks® assay binds free Pyd and Dpd while the antibody for the Pyrilinks®-D assay binds only free Dpd. Therefore figure 1b indicates that the contribution of Dpd to the Pyd + Dpd data (Figure 1a) is relatively small (Dpd ratios of 10 to 35 vs. Pyd + Dpd ratios of 50 to 250 nmol / Creatinine mmol). This means that the contribution of bone (Dpd) to lactation crosslinks excretion in the sow is minor compared to crosslinks from non-bone tissues (Pyd). Based on the mean excretion ratios in Table 1, there was approximately 12 times more Pyd being released than Dpd. Even though Pyd is not uterine-specific, the amount and rate of collagen degradation that occurs during involution makes the uterus a likely source of this excess Pyd.

Table 1. Litter and crosslinks excretion statistics of 5 Yorkshire sows that farrowed on the same day and lactated 21 d. Abbreviations: sow weaning-to-estrus interval (WEI), standard deviation (S.D.), pyridinoline (Pyd), deoxypyridinoline (Dpd) and creatinine (Cre).

Sow ID	888R	927G	948G	033R	025R
Parity	2	2	1	1	1
Total Born	13	8	13	12	11
Born Alive	13	7	13	12	11
Still Born	0	1	0	0	0
Mummies	0	0	1	0	0
Litter Wt. 2 d, kg	19.4	15.7	21.4	16.3	16.0
Pigs Weaned	10	10	8	11	10
Litter Wt. Weaned, kg	66.6	77.2	60.0	63.2	68.7
Sow Wt. at Wean, kg	186.3	178.6	146.5	154.0	138.6
Sow WEI, d	4	5	5	5	7
Mean Pyd + Dpd / Cre, nmol / mmol	115.2	135.0	155.6	112.3	165.2
S.D. Pyd + Dpd / Cre, nmol / mmol	45.2	52.7	52.1	28.8	64.4
Mean Dpd / Cre, nmol / mmol	5.8	16.2	12.2	8.6	13.6
S.D. Dpd / Cre, nmol / mmol	1.5	8.0	3.6	1.5	4.1





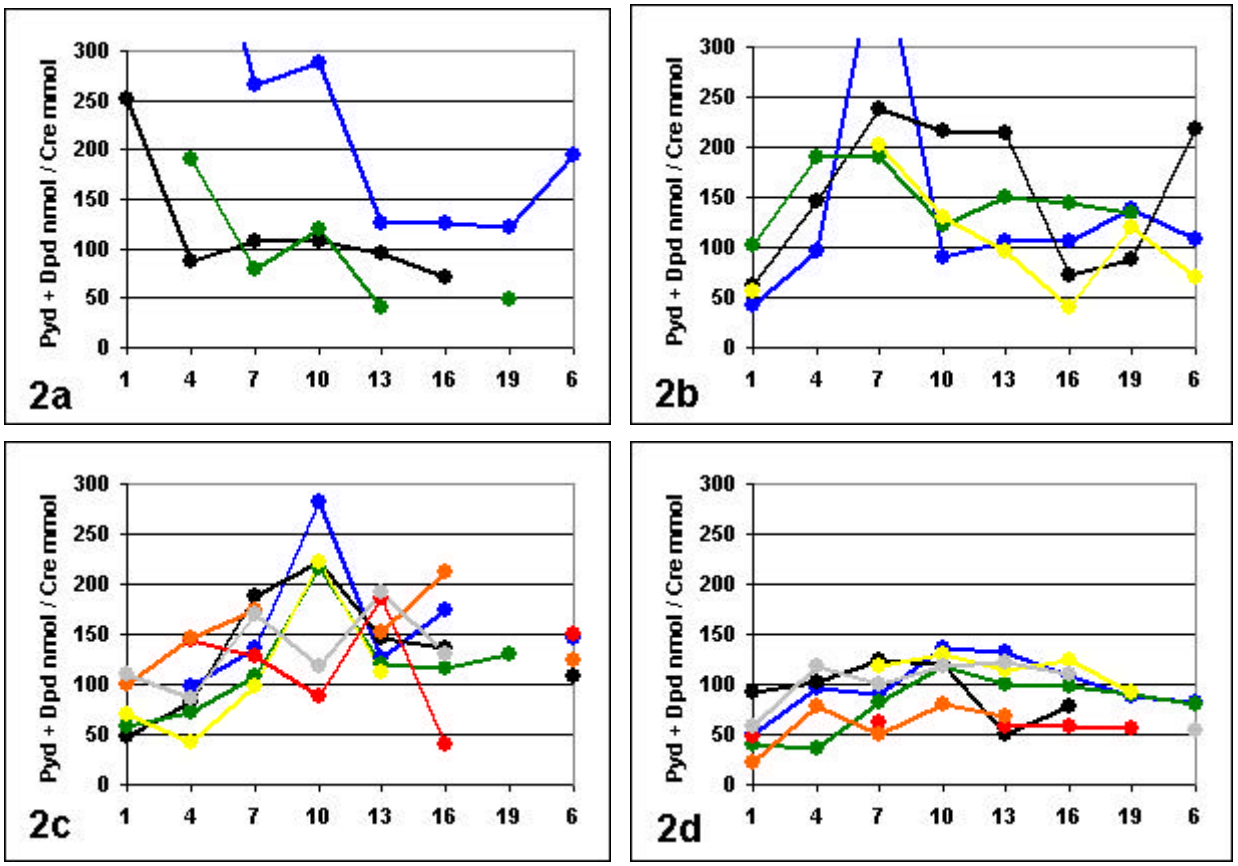
Figures 1a, 1b. Urinary excretion of free Pyd and Dpd (top, Pyrilinks® assay) and free Dpd alone (bottom, Pyrilinks-D® assay) in five sows around farrowing, during lactation (1 to 21 d), and postweaning (2 to 8 d). Vertical dashed lines mark farrowing (0 d) and weaning (21 d).

Materials and Methods (Experiment II)

A second study, involving more sows, was conducted based on the variation in Pyd and Dpd ratios between the five sows studied in Experiment I. Urine samples were collected from 21 multiparous sows every 3 days through lactation (1, 4, 7, 10, 13, 16, 19 d) and then once postweaning (6 d). Again, every attempt was made to collect samples between 0630 and 0830 h but approximately 2/3 of the samples were obtained later in the day, between 0830 and 1530 h. Sow management, urine collection and assays were the same as in Experiment I except that sows farrowed over a 4 to 5 d period so lactation lengths ranged from 18 to 23 d. In addition, sows were split-weaned with

2 to 4 pigs weaned at 14 d and the remainder weaned at 21 d. Intra and inter-assay coefficients of variation were < 5% for all three assays. Each sow's crosslinks data was categorized by when peak Pyd + Dpd excretion occurred during lactation. A peak was defined as the maximum Pyd + Dpd ratio that exceeded the population mean ratio by 2 standard deviations (119.6 + 2 (38.8)). Sows exhibited one of four crosslinks excretion patterns: peak on 1 or 4 d (early, n = 3), peak on 7 d (mid, n = 4), peak on 10, 13, or 16 (late, n = 7) or no peak (none, n = 7). Differences in sow and litter variables between these four classes were examined with a general linear models (GLM) analysis (SAS, 1990).

Results and Discussion (Experiment II)



Figures 2a, 2b, 2c, 2d. Urinary excretion of free Pyd and Dpd (Pyrilinks® assay) in lactating sows that peaked on 1 or 4 d (early, n = 3, top-left), on 7 d (mid, n = 4, top-right), on 10, 13 or 16 d (late, n = 7, bottom-left) or did not peak (none, n = 7, bottom-right). X-axis is day of lactation (1 to 19 d) and postweaning (6 d).

Excretion of Dpd (Pyrilinks®-D assay) remained low (≤ 30 Dpd nmol / Cre mmol, graphed data not shown) relative to Pyd as in experiment I. The data on Pyd + Dpd excretion (Figures 2a, 2b, 2c, 2d, Pyrilinks® assay) revealed a greater amount of variation between sows than in experiment I. A group of sows (n = 7) showed no significant increase or peak in Pyd + Dpd excretion during lactation and remained below a ratio of 150 Pyd + Dpd nmol / Cre mmol (Figure 2d). Another group of sows (n = 3) had very high Pyd + Dpd ratios immediately post-farrowing that fell rapidly at different points (Figure 2a). The remaining 11 sows in the study exhibited a significant peak in Pyd

+ Dpd excretion during mid (n = 4) or late (n = 7) lactation (Figures 2b, 2c). There were several differences in sow and litter lactation performance variables between these four classes (Table 2) but, none of them seemed to offer a sufficient explanation for the radical differences in crosslinks excretion. There tended to be an effect of peak class on the number of pigs suckled after 2d (P < .08) because sows in the early peak class suckled more pigs than those in the late class (P < .02). This difference in litter size suckled must have resulted from cross fostering since it is not reflected in the number of pigs born alive. There was also an effect of peak class on the number of pigs weaned at 14 d and weight of pigs weaned at 14 d (P < .01). Sows in the mid peak class had more pigs and thus more litter weight weaned at 14 d than either the late or no peak classes (P < .01). These pigs were removed a week early to supply a different experiment. Peak class affected litter weight weaned at 21 d (P < .02) because sows in the no peak class had a lower 21 d litter weight than those in the late peak class (P < .01) even though there were no differences in number of pigs

Table 2. Effect of time of peak lactation Pyd + Dpd excretion (peak class) on litter and crosslinks excretion statistics of 21 multiparous sows. Data presented as LS means ± SEM. Abbreviations: standard deviation (S.D.), pyridinoline (Pyd), deoxypyridinoline (Dpd) and creatinine (Cre).

Peak Class **Peak Day**	**Early** **1, 4**	**Mid** **7**	**Late** **10, 13, 16**	**None**	**Class Effect**
Number of Sows	3	4	7	7	
Lactation Length, d	21.3±0.9	22.5±0.7	20.9±0.5	21.1±0.5	P > .37
Parity	3.3±1.5	2.0 ± 1.3	4.6 ± 1.0	4.1 ± 1.0	P > .47
Pre-Farrow Sow Wt., kg	240.4±14.9	217.1±12.9	229.9±9.7	231.0±9.7	P > .69
Total Born	10.7±2.0	11.3±1.7	11.9±1.3	11.3±1.3	P > .96
Born Alive	10.7±1.8	10.3±1.5	8.7±1.2	10.7±1.2	P > .63
Still Born	0.0±1.5	1.0±1.3	3.1±1.0	0.6±1.0	P > .22
Mummies	0.3±0.2	0.0±0.2	0.3±0.1	0.0±0.1	P > .31
Litter Wt. 2 d, kg	15.0±1.7	17.0±1.5	14.1±1.1	14.7±1.1	P > .51
Pigs Suckled	12.0±0.8	10.8±0.7	9.4±0.5	10.4±0.5	**P < .08**
Pigs Weaned 14 d	3.7±0.7	4.8±0.6	2.3±0.4	2.1±0.4	**P < .01**
Litter Wt. Weaned 14 d, kg	17.5±3.6	24.1±3.1	10.1±2.4	10.0±2.4	**P < .01**
Pigs Weaned 21 d	5.3±0.3	5.3±0.3	5.6±0.2	5.1±0.2	P > .59
Litter Wt. Weaned 21 d, kg	38.2±1.9	38.0±1.6	40.9±1.2	34.5±1.2	**P < .02**
Total Pigs Weaned	9.0±0.8	10.0±0.7	7.9±0.5	7.3±0.5	**P < .03**
Sow Wt. at Wean, kg	206.5±14.6	207.3±12.6	205.4±9.5	211.1±9.5	P > .97
Sow Wt. Change, kg	-33.9±9.7	-9.9±8.4	-24.5±6.3	-19.8±6.3	P > .30
Sow Wt. Change Adj.*, kg	-18.9±9.3	7.2±8.0	-10.4±6.1	-5.1±6.1	P > .20
Mean Pyd+Dpd/Cre, nmol/mmol	149.2±18.1	135.9±15.7	132.5±11.9	84.6±11.9	P < .02
S.D. Pyd+Dpd/Cre, nmol/mmol	88.6±12.9	69.1±11.2	53.4±8.4	24.2±8.4	P < .01
Mean Dpd/Cre, nmol/mmol	20.5±3.4	14.9±2.9	14.5±2.2	9.6±2.2	P < .09
S.D. Dpd/Cre, nmol/mmol	16.4±2.1	5.7±1.8	5.1±1.4	3.8±1.4	P < .01
* Sow weight change from pre-farrow to weaning adjusted for litter weight at 2 d					

weaned at 21 d (P > .5). There was also a peak class effect on total pigs weaned (14 d + 21 d, P < .03) because sows in the late and no peak classes weaned fewer total pigs than those in the mid peak class (P = .02). None of these differences in number of pigs weaned or weight of pigs weaned at 14 or 21 d are likely to be the cause of the different crosslinks excretion patterns because they occurred after, not before, the changes in crosslinks excretion. We had expected sow variables like litter size, parity, weight or weight change to affect crosslinks excretion but there was no evidence of this. In theory, sows that gestated the largest litters should have deposited the

most uterine collagen and had the most collagen to degrade and crosslinks to excrete. However, there was no significant correlation between total born and any of the Pyd + Dpd statistics that would support such a relationship.

Based on data from postpartum rats (Gunja-Smith, 1989), cows (Kaidi et al., 1991; Liesegang et al., 2000) and humans (Stone and Franzblau, 1995) we had anticipated that Pyd would be a good marker of uterine collagen degradation in the sow. There are several possible factors that could explain why the excretion of Pyd seems to be so unpredictably variable in our present studies. One key difference between our studies and those in other species cited above is that we used EIA assays to measure free (non-protein bound) crosslinks instead of high performance liquid chromatography (HPLC) that estimates total crosslinks (protein bound and non-protein bound). Human literature suggests that the ratio of free to bound crosslinks (≈ 2:3) does not change but, to our knowledge, this concept has not been tested in swine. If the ratio of free to bound crosslinks were to change during the periods we studied it could easily mask changes in crosslinks excretion. The EIAs we utilized had acceptable levels of intra and inter-assay variation but the inconsistent time of sample collection could have added variation since there is some diurnal variation in crosslinks excretion. Another factor to consider is that the epitheliochorial placentation of porcine embryos is a loose and non-invasive type of attachment compared to the more invasive placentation of rats, cattle and humans. This important species difference may impact the collagen degradation and crosslinks excretion pattern during uterine involution. A final possibility is that the radically different crosslinks excretion patterns between sows may represent 'true' biological variation. Since Pyd is not uterine-specific, other non-bone tissues could be significantly contributing to the crosslinks excretion pool. Our current focus involves investigating the possible sources of the variation between sows discussed above and in developing a repeatable and direct physical measure of uterine involution to compare to the collagen degradation marker data.

Implications

The development of an accurate non-invasive means to monitor uterine involution would give researchers a tool to study the effects of uterine involution on subsequent embryo survival. Such a tool might also provide producers with information on sow reproductive ability to base management decisions on. The present data suggest that pyridinoline, a marker of collagen degradation, is excreted by lactating sows in a pattern consistent with the rapid and extensive loss of collagen that occurs during uterine involution. However, several sows failed to exhibit a significant increase in pyridinoline excretion during lactation and factors that might explain this difference are being investigated.

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[1] Department of Poultry Science