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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan  
Mob: +92 300 3008585, Fax: +92 41 8815544  
E-mail: editorijps@gmail.com

## Gender, Age and Reproductive Status Effects on Serum Prolactin Concentrations in Different Varieties and Species of Poultry

F.W. Edens

Department of Poultry Science, North Carolina State University, Raleigh, North Carolina 27695-7635, USA

**Abstract:** Homologous chicken prolactin (chPRL) and turkey prolactin (trPRL) radioimmunoassays were used to assess the effects of gender, age and reproductive status on serum prolactin concentrations in several varieties and species of poultry. Adult males (chickens, turkeys [domestic and wild], Coturnix quail and chukar partridges) had lower serum prolactin (PRL) concentrations than did their female counterparts. Aging in meat-type female chickens appeared to be associated with increasing PRL concentrations, but aging of White Leghorn chickens did not result in increased serum prolactin concentrations. Aging in males did not alter serum prolactin concentrations. In female chukar partridges, serum prolactin concentrations were similar to that of White Leghorn hens and male chukar serum prolactin concentrations were similar to levels found in male chickens. Serum prolactin concentrations in Coturnix males and females, with females having greater serum prolactin than males, were generally higher than in males and females of other fowl involved in this study. Broodiness was associated with greatly elevated serum prolactin concentrations. The serum prolactin concentrations derived from the use of the chPRL assay as a heterologous assay for chukar and domestic and wild turkeys, yielded results that were lower but in parallel with results from trPRL assay. The homologous chicken PRL radioimmunoassay appeared to be useful as a heterologous assay for domestic and wild turkey and for chukar partridge PRL.

**Key words:** Prolactin, chicken, turkey, chukar, Coturnix, gender, age

### INTRODUCTION

Prolactin (PRL) has been isolated from pituitaries of chickens (Scanes *et al.*, 1975) and turkeys (Burke and Papkoff, 1980). It has been reported to be an inducer of broodiness in turkey and chicken hens as indicated by increased PRL concentrations in circulation coincidentally with nesting behavior and the onset of broodiness (Burke and Dennison, 1980; El Halawani *et al.*, 1980a,b; Harvey and Bedrak, 1984; Proudman and Opel, 1981; Riddle *et al.*, 1935; Sharpe *et al.*, 1988). Administration of PRL terminates egg laying and induces ovarian regression but is not always accompanied by incubation behavior in chickens and turkeys (Opel and Proudman, 1980). However, Hargis *et al.* (1987) gave ovine PRL to turkey hens causing ovarian regression and induction of incubation behavior. The decreased egg production, during times of elevated plasma PRL coincidentally occurring with the development of broody behavior, could be attributed to the anti-gonadal role of PRL (Opel and Proudman, 1980).

In addition to the widely-believed PRL-influence on reproductive behavior, Goldsmith and Follett (1980) have indicated that there are other functions for prolactin in birds. These functions include induction of hypertrophy of pigeon crop sac and crop milk production, development of the brood patch, induction of lipogenesis (Goodridge and Ball, 1967a,b), involvement in acute stress responses (Opel and Proudman, 1984; Chastel

*et al.*, 2005), modulation of the immune system (Skwarlo-Sonta *et al.*, 1987), osmoregulation (Harvey *et al.*, 1979a) and decreased rate of food intake in photostimulated turkeys (Denbow, 1986).

In both research and commercial settings, there are increasing needs to assess serum PRL concentrations as part of an overall managerial effort to segregate reproductively active hens from those hens entering the nonproductive broody status in flocks of turkeys, broiler breeders, game birds and possibly table egg layers in these different sectors of the poultry industry. There are limited data dealing with species, sex, varietal and reproductive status relationship to serum PRL in domestic birds. The objectives of this investigation were to look, primarily, at serum PRL concentrations in several varieties of domestic and wild fowl in either productive or nonproductive (broody) status and, secondarily, to evaluate the usage of a homologous chicken PRL (chPRL) radioimmunoassay that might be used to assess nonproductive (broody) status in turkeys. Additionally, attempts were made to measure serum PRL in other species of fowl using the chPRL/turkey (tr)PRL radioimmunoassay.

### MATERIALS AND METHODS

**Birds:** All of the birds used in this study were obtained from the North Carolina Agricultural Research Service Poultry Research Unit at North Carolina State University and were in projects approved by the Institutional Animal

Care and Use Committee. The different varieties and species of poultry were maintained for the academic affairs program and ages of the different varieties and species used were variable. All birds, with the exception of Coturnix quail, which were maintained in mating pairs in egg laying cages, were maintained as mixed-gender flocks in litter-covered floor pens in curtain-side wall poultry barns with fan ventilation. Reproductive status of the birds was determined by daily observation before blood samples were collected. Broody birds were determined by their persistent nesting and rapid return to the nest if removed. Birds in active egg production were determined by observation and by palpation of the shell gland. Venous blood samples were taken from hens only if there was no egg in the shell gland. After anatomical sexing of the male and female chukar partridges, the females were examined to determine their egg producing status and only females in egg production were selected for blood sampling. There were no nonproductive chukar females in the flock. With exception of the juvenile male and pullet broiler chickens (4 weeks old), all males (chicken, turkey, chukar and Coturnix) were reproductively mature. Mature male and female chickens (White Leghorn, Rhode Island Red, Barred Plymouth Rock and White Plymouth Rock) were 30-42 (young) to 60-110 (old) weeks old, the chukars were approximately 60 weeks old, the wild turkeys were approximately 2 years old. Age of the domestic turkey hens was approximately 52 weeks and the age of the Coturnix quail was approximately 12 weeks. All the birds were on the same photostimulatory light:dark (18hr:6hr) cycle and feed, appropriate for the reproductive status of each species and fresh water were provided on an *ad libitum* basis.

**Blood and serum collection:** Blood samples were collected in the morning between 0900 and 1100 hr to prevent confounding of data due to a reported blood prolactin diurnal increase during the late afternoon (Bedrak *et al.*, 1981). All samples were collected from the birds in early June and ambient temperatures ranged between 20°C and 27°C.

Venous blood samples were collected from the ulnar wing vein of all birds except for the Coturnix quail, which were bled via the jugular vein. Volumes not exceeding 2 mL were collected from all birds except the Coturnix quail from which only 1 mL per bird was collected. Venous blood was collected in sterile plastic syringes and was then transferred into polycarbonate tubes and allowed to coagulate for a period of two hours in a 4°C environment for expression of serum. After the serum was expressed, each blood tube was ringed and serum was decanted into 2 mL microcentrifuge tubes, sealed and frozen at -80°C until assayed for prolactin.

**Prolactin (PRL) assay:** Chicken prolactin (chPRL) was determined in domestic chickens (*Gallus domesticus*) by homologous Radioimmunoassay (RIA) previously

described by Edens and Parkhurst (1994). Purified chPRL (AFP-10328B: Dr. A. F. Parlow, Pituitary Peptide Hormones and Antisera Center, Harbor-UCLA Medical Center, Torrance, California 90509) was used as a standard and the chPRL antigen (AFP-444B) was iodinated with <sup>125</sup>I (Salacinski *et al.*, 1981). Rat anti-chPRL antisera (AF-1622288) and goat anti-rat gamma globulin were used as primary and secondary antibodies in the chPRL radioimmunoassay. The chPRL was used also as a heterologous RIA to determine serum PRL concentrations in turkeys (domestic and wild [*Meleagris gallopavo*]), chukar partridges (*Alectoris graeca chukar*) and Coturnix (*Coturnix coturnix japonica*) quail. A radioimmunoassay specific for trPRL (Proudman and Opel, 1981) was used to determine trPRL in domestic and wild turkey hens (in production and broody) and in domestic and wild turkey toms. Turkey PRL-II (supplied by Dr. John Proudman, US Department of Agriculture, Beltsville, MD) was used as the antigen and was iodinated with <sup>125</sup>I (Salacinski *et al.*, 1981). Antibody (ovine anti-rabbit gamma globulin) solutions were provided by Dr. Proudman. Eighty-five percent binding in the trPRL radioimmunoassay occurred at 51 ng/mL and 15% binding occurred at 1080 ng/mL. The trPRL concentrations were determined by a single radioimmunoassay with an intra-assay coefficient of variation of 2.43%.

Inhibition curves were generated for serially diluted chPRL and trPRL serum and for chPRL and trPRL standards. The inhibition curves were linearized using a logit transformation and slopes were then determined. The slopes for the serially diluted chicken and turkey standard and serum samples were 2.15, 2.41 and 2.32, respectively. The slopes for serially diluted chukar and Coturnix serum samples and chPRL standard were 2.37, 2.46 and 2.15, respectively. Nonspecific binding tubes (<sup>125</sup>I-chPRL plus buffer) and B<sub>0</sub> tubes (antibody, buffer and <sup>125</sup>I-chPRL/trPRL) were used to detect nonspecific binding and maximum binding. Maximum binding was 57.1%, 34.6%, 43.9% and 37.8% for chicken, turkey, chukar and Coturnix serum, respectively. The lowest detectable concentration of either chPRL or trPRL was 0.8 ng/mL. The chPRL and trPRL concentrations in serum were determined by a single radioimmunoassay and the intra-assay coefficient of variation was 1.33% (homologous RIA) and 5.8% (heterologous RIA). All assays were conducted in quadruplicate tubes containing 50 µl of serum. Each assay tube contained approximately 30,000 cpm of <sup>125</sup>I-chPRL or <sup>125</sup>I-trPRL.

**Data analysis:** A completely randomized experimental design was used in this study. All data were subjected to analysis of variance using the general linear models procedure of the Statistical Analysis System (SAS, 1996). Statements of significance are based on  $p \leq 0.05$ . When there were significant differences, means were separated by least significant difference (SAS, 1996).

Table 1: Serum prolactin (ng/mL) as determined with the chicken prolactin assay in young (30-42 weeks old) and old (60-110 weeks old) chicken hens in egg production and broody chicken hens from different varieties

Variety	Prolactin (ng/mL)	
	Young	Old
Leghorn <sup>1</sup>	19.49±2.73 <sup>a,c</sup>	24.92±1.74 <sup>a,d</sup>
White Plymouth Rock	25.30±2.51 <sup>a,c</sup>	34.66±2.44 <sup>b,c</sup>
Rhode Island Red	9.97±1.76 <sup>d</sup>	66.99±4.91 <sup>c,b</sup>
Rhode Island Red (Broody)	179.68±15.93 <sup>b,a</sup>	328.66±28.17 <sup>a</sup>
Barred Plymouth Rock	23.77±4.20 <sup>d,c</sup>	49.33±13.84 <sup>b,c</sup>
Barred Plymouth Rock (Broody)	64.20±12.75 <sup>b,b</sup>	144.36±11.35 <sup>a</sup>

<sup>1</sup>N = 12 for each mean±SE except for Rhode Island Red (Broody) and Barred Plymouth Rock (Broody) which were represented by 5 and 4 hens, respectively.

<sup>a,b,c,d</sup>Means±SE, within a variety, with unlike lower case superscripts differ significantly (p≤0.05).

<sup>A,B,C</sup>Means±SE within ages among varieties with unlike upper case superscripts differ significantly (p≤0.05)

## RESULTS

Shown in Table 1 are the mean concentrations of serum PRL in young and old laying hens of different varieties and the values of serum PRL in young and old broody hens in the Rhode Island Red and Barred Plymouth Rock varieties. There were significant varietal and age effects, and there was an effect for reproductive status also. Old Rhode Island Red, White Plymouth Rock and Barred Plymouth Rock varieties had higher concentrations of PRL than did their younger counterparts, but there was no difference between serum PRL concentrations in young and old White Leghorn hens. Table 1 also shows the within age differences in serum PRL among chicken varieties. The young Leghorn serum PRL concentrations were significantly higher than the Rhode Island Red concentrations but did not differ from the White Plymouth Rock and Barred Plymouth Rock serum PRL concentrations. In the older hens, the Barred Plymouth Rock PRL concentrations did not differ significantly from the Rhode Island Red concentrations and these were significantly higher than the White Plymouth Rock concentrations, which were higher than White Leghorn PRL concentrations. Old broody hens had serum PRL concentrations that were significantly higher than serum PRL concentrations among their younger broody counterparts.

The PRL concentrations in adult male chickens are shown in Table 2 and there was a varietal effect but not an age effect. Young Leghorn males had significantly higher PRL concentrations than did White Plymouth Rock males. PRL concentrations in young Rhode Island Red and Barred Plymouth Rock males were intermediate to the concentrations in Leghorn and White Plymouth Rock males. In older males, there were no differences among the four different varieties and there were no differences due to age among these four varieties. Nevertheless, with exception of the White Leghorn males, the older roosters among the Barred

Table 2: Serum prolactin (ng/mL) as determined with the chicken prolactin assay in young (30-42 weeks old) and old (60-110 weeks old) reproductively active male chickens from different varieties

Variety	Prolactin (ng/mL)	
	Young	Old
Leghorn <sup>1</sup>	8.90±0.64 <sup>a,A</sup>	7.79±0.83 <sup>a,A</sup>
Rhode Island Red	7.05±0.51 <sup>ab,A</sup>	8.11±1.09 <sup>a,A</sup>
Barred Plymouth Rock	5.93±0.49 <sup>ab,A</sup>	7.73±0.96 <sup>a,A</sup>
White Plymouth Rock	5.05±0.33 <sup>b,A</sup>	6.54±0.86 <sup>a,A</sup>

<sup>1</sup>N = 8 for each mean±SE.

<sup>ab</sup>Means±SE with unlike lower case superscripts are significantly different (p≤0.05).

<sup>A</sup>Means±SE with the same upper case superscript do not differ (p>0.05)

Plymouth Rock, Rhode Island Red and White Plymouth Rock males showed indications of general elevation in serum PRL associated with aging.

Comparisons of PRL concentrations in different species of fowl, fowl in different reproductive status and determinations of PRL in turkeys using a homologous assay for turkey PRL compared to the radioimmunoassay for chPRL are presented in Table 3. Broiler cockerels and pullets, under the stress induced by a natural *Eimeria tenella* parasitism in the intestinal tract (Table 3), had concentrations of PRL that were lower than their White Plymouth Rock adult counter-parts (Table 3). However, a stressor such as that caused by a coccidial infection did not cause any alteration in serum PRL concentrations compared with normal, uninfected cockerels and pullets (Table 2).

Male Coturnix and male chukar partridges had significantly (p≤0.05) lower PRL concentrations than did the Coturnix and chukar females. It was of interest that when chukar serum was assayed by trPRL RIA, both male and female serum PRL concentrations were generally greater than when assayed with the chPRL RIA (Table 3).

There were significant differences when domestic and wild turkey serum PRL concentration results from chPRL vs. trPRL RIA were compared for females in egg production, broody hens and males (Table 3). Generally, serum PRL concentrations were significantly lower when the chPRL was used for the assay. The lone exception was for the male wild turkey serum PRL concentrations in which the results from chPRL were lower but not significantly compared with trPRL (Table 3). In this assay, turkey PRL did bind to chicken PRL antibody, but the slope of the dose response curve for turkey serum was flatter than the chPRL reference preparation. Thus, under these conditions, the accuracy of the measurement was affected as can be seen with inspection of the data presented in Table 3. Adult domestic turkey hens in egg production had significantly (p≤0.001) lower PRL concentrations than did broody domestic turkey hens when the PRL determinations

Table 3: Serum prolactin (ng/mL) as determined with the chicken prolactin assay or with the turkey prolactin assay from different species of fowl differing in age and reproductive status

Species and reproductive condition	Prolactin (ng/mL)
Juvenile male broiler chickens, normal <sup>1</sup>	6.65±0.39 <sup>a</sup>
Juvenile male chickens with coccidiosis <sup>1</sup>	5.71±0.69 <sup>a</sup>
Juvenile female broiler chickens, normal <sup>1</sup>	8.24±0.55 <sup>a</sup>
Juvenile female broiler chickens with coccidiosis <sup>1</sup>	9.33±0.74 <sup>a</sup>
Mature male Coturnix quail <sup>1</sup>	11.21±1.97 <sup>b</sup>
Mature female Coturnix quail <sup>1</sup>	49.07±9.65 <sup>a</sup>
Mature male chukar partridge <sup>1</sup>	4.33±0.15 <sup>a</sup>
Mature female chukar partridge <sup>1</sup>	19.49±2.45 <sup>b</sup>
Mature male chukar partridge <sup>2</sup>	8.45±3.06 <sup>bc</sup>
Mature female chukar partridge <sup>2</sup>	32.67±4.59 <sup>a</sup>
Adult domestic turkey hen in egg production <sup>1</sup>	35.00±1.22 <sup>b</sup>
Adult domestic turkey hen in egg production <sup>2</sup>	105.23±9.33 <sup>a</sup>
Broody domestic turkey hen <sup>3</sup>	165.69±68.12 <sup>b</sup>
Broody domestic turkey hen <sup>4</sup>	1079.28±125.67 <sup>a</sup>
Adult domestic turkey tom <sup>5</sup>	6.03±1.48 <sup>b</sup>
Adult domestic turkey tom <sup>6</sup>	10.19±1.66 <sup>a</sup>
Adult wild turkey hen in egg production <sup>7</sup>	25.29±11.59 <sup>d</sup>
Adult wild turkey hen in egg production <sup>8</sup>	133.47±17.95 <sup>c</sup>
Broody wild turkey hen <sup>9</sup>	251.00±39.86 <sup>b</sup>
Broody wild turkey hen <sup>10</sup>	579.33±73.00 <sup>a</sup>
Adult wild turkey tom <sup>11</sup>	3.21±1.08 <sup>a</sup>
Adult wild turkey tom <sup>12</sup>	7.49±1.66 <sup>a</sup>

<sup>1</sup>N = 15 for each mean±SE. The chPRL assay was used to make these measurements.

<sup>2</sup>N = 15 for each mean±SE. The trPRL assay was used to make these measurements. These serum samples were the same as those labeled with the superscript 1.

<sup>3</sup>N = 10 for each mean±SE. The chPRL assay was used to make these measurements.

<sup>4</sup>N = 10 for each mean±SE. The trPRL assay was used to make these measurements. These serum samples were the same as those labeled with the superscript 3.

<sup>5</sup>N = 10 for each mean±SE. The chPRL assay was used to make these measurements.

<sup>6</sup>N = 10 for each mean±SE. The trPRL assay was used to make these measurements. These serum samples were the same as those labeled with the superscript 5.

<sup>7</sup>N = 10 for each mean±SE. The chPRL assay was used to make these measurements.

<sup>8</sup>N = 10 for each mean±SE. The trPRL assay was used to make these measurements. These serum samples were the same as those labeled with the superscript 7.

<sup>9</sup>N = 3 for each mean±SE. The chPRL assay was used to make these measurements.

<sup>10</sup>N = 3 for each mean±SE. The trPRL assay was used to make these measurements. These serum samples were the same as those labeled with the superscript 9.

<sup>11</sup>N = 4 for each mean±SE. The chPRL assay was used to make these measurements.

<sup>12</sup>N = 4 for each mean±SE. The trPRL assay was used to make these measurements. These serum samples were the same as those labeled with the superscript 11.

<sup>a,b,c,d</sup>Within a fowl species, mean±SE with unlike lower case superscripts differ significantly ( $p \leq 0.05$ )

were made using either the chPRL assay or the trPRL assay. Adult wild turkey hens in egg production were found to have lower PRL concentrations, and when these same samples were analyzed with the trPRL assay, the wild turkey hens had PRL concentrations similar to those of their domestic counter-parts (Table 3).

## DISCUSSION

The use of a homologous chPRL radioimmunoassay for the measurement of chicken PRL is accurate and repeatable in chickens and this was shown by a mean intra-assay variation of only 1.33% for the chicken samples. Mean intra-assay variation for the trPRL assay was 2.43% showing its specificity for turkey PRL. The concentrations of serum PRL in young and old laying hens (Table 1) fell within a range of values reported by several groups of scientists measuring chicken PRL. Sharpe *et al.* (1988) reported laying bantam hens to have PRL concentrations near 30 ng/mL but broody bantams to have concentrations between 90 and 105 ng/mL and values ranging between 64 to 144 ng/mL for broody Barred Plymouth Rock and 180 to 329 ng/mL for broody Rhode Island Red hens, respectively, were found in this study. Bedrak *et al.* (1981) reported concentrations of serum PRL to be 16.4 and 51.1 ng/mL in laying and broody White Plymouth Rock hens, respectively, and in this study, young and old White Plymouth Rock hens had serum PRL concentrations ranging between 25 and 35 ng/mL. Harvey *et al.* (1979a) reported adult chicken hens from a layer strain to have PRL concentrations around 122 ng/mL. Furthermore, Harvey *et al.* (1979a) observed that females had decreasing PRL concentrations during the growth period but showed an increasing trend in PRL concentrations during the pre-laying period. In this report, there appeared to be increasing PRL concentrations associated with increasing age in all four varieties of female chickens examined.

Although there are limited data dealing with circulating PRL in chickens, the data from this report and others, cited herein, indicate that there may be considerable variation in PRL concentrations among different varieties of domestic chickens. Additionally, as laying chickens age, there may be a concomitant increase in circulating concentrations of PRL which possibly could be involved in a natural decrease in the rate of lay due to an anti-gonadal role played by PRL (Opel and Proudman, 1980). The influence of age in males on serum PRL is not so clear, but based on slightly but not significantly elevated serum PRL in older males, it might be conceivable that the anti-gonadal effect of PRL has little influence in the male chicken and possibly even in other species of fowl (Table 3).

Levels of plasma PRL have been reported to be in the range of 10 to 40 ng/mL in 8 and 3 weeks old cockerels, respectively (Hall *et al.*, 1985), but PRL concentrations in cockerels from a layer strain have been reported to decrease from 155 to 37 ng/mL from hatch to 24 weeks of age (Harvey *et al.*, 1979a) and in a broiler strain to decrease from 226 to 90 ng/mL from 3 to 8 weeks of age (Harvey *et al.*, 1979b). In this study, four weeks old broilers, under the stress of a coccidiosis parasitism,

were found to have PRL concentrations around 5.7 and 9.3 ng/mL in cockerels and pullets, respectively. In normal male and pullet broilers, serum PRL ranged between 6.7 and 8.2 ng/mL, respectively. However, stress, such as that associated with water deprivation in broiler chickens, has not been reported to affect baseline serum prolactin concentrations (Harvey *et al.*, 1979a). There is an obvious disparity among the reported observations of Harvey *et al.* (1979a,b), Hall *et al.* (1985) and the data reported here. In this report, concentrations of serum PRL in roosters (Table 2) were determined to range between 5.05 and 8.90 ng/mL and these concentrations were significantly less than the concentrations reported for roosters from a layer strain of chicken (Harvey *et al.*, 1979a).

The PRL concentrations among the four varieties of roosters were found to be somewhat variable (Table 2), but they generally did not change significantly in response to aging. It is important to note that the male PRL concentrations (Table 3) were significantly lower than in their female counterparts, a condition similar to that reported by Harvey *et al.* (1979a). It was of interest to note, in this investigation, that the highest concentration of serum PRL was in Leghorn roosters followed by Rhode Island Red, Barred Plymouth Rock and White Plymouth Rock in decreasing order of concentrations. This trend, showing lower concentrations of PRL in meat production varieties of chickens than in egg producing varieties of chickens, was similar to the findings of Harvey *et al.* (1979a).

There was cross reactivity of the rat anti-chPRL antibody with serum PRL from domestic turkeys, wild turkeys, chukar partridges and Coturnix quail (Table 3). Circulating PRL values have been reported in turkey hens ranging from 10 to 16 ng/mL (laying and broody; Harvey *et al.*, 1981), 20 to 709 ng/mL (laying to broody, respectively; Wentworth *et al.*, 1983), 28 to 81 ng/mL (laying to broody, respectively; Zadworny *et al.*, 1985), 42 to 377 ng/mL (laying to broody, respectively; Proudman and Opel, 1981), to 361 to 1602 ng/mL (laying to broody, respectively; Burke and Papkoff, 1980). In this report, laying turkey hen serum PRL was measured, using a chPRL radioimmunoassay and was found to be 35 to 166 ng/mL (domestic laying to domestic broody, respectively) and 25 to 251 ng/mL (wild laying to wild broody, respectively), but using the radioimmunoassay for trPRL (Proudman and Opel, 1981) in the same samples, domestic turkey hen PRL concentrations were 105 to 1079 ng/mL (laying to broody, respectively) and 133 to 579 ng/mL for the laying and broody wild turkey hen. Adult domestic male turkey serum PRL concentrations were 6.0 and 10.2 ng/mL for chPRL and trPRL assays, respectively. Adult male wild turkey PRL was found to be approximately 3.21 ng/mL with the chPRL assay and was 7.49 ng/mL using the trPRL assay. The adult domestic and wild male serum PRL concentration, measured with the chPRL and trPRL

assays, were in agreement with published values of 5.20 to 7.50 ng/mL for adult male domestic turkeys (Proudman and Opel, 1981; Opel and Proudman, 1984). Coturnix male and female serum PRL concentrations, as measured by the chPRL assay were generally greater than similar results from chickens and turkeys in a similar reproductive status (Table 1, 2 and 3). Results from chPRL and trPRL assays for serum prolactin in male and female chukars were different also (Table 3), but the results from the chPRL assay, although generally lower in concentration, were in parallel with the results from the trPRL assay (Table 3).

It is obvious that there are many different PRL values being reported for laying and broody turkey hens. However, each citation of a trPRL radioimmunoassay, made herein, represents independent assay development and these may have used different purified turkey PRL isoforms. Thus, a different primary antibody would have been used in the respective assays. Prolactin, similar to many neuropeptide hormones can be described as a complex hormone made up of many isoforms which can aggregate to form dimers and larger molecules (Scanes *et al.*, 1975; Burke and Papkoff, 1980; Proudman and Corcoran, 1981; Corcoran and Proudman, 1991). Prolactin in the different isoforms may have different biological properties (Proudman and Corcoran, 1981; Corcoran and Proudman, 1991) and their ratios may be altered under different environmental and physiological conditions such as environmental stressors, water deprivation, nest deprivation, presence of eggs, tactile stimulation of the skin of the breast, presence of chicks or poults and the species of the bird (El Halawani *et al.*, 1980a,b; Goldsmith and Hall, 1980; Goldsmith and Williams, 1980; Hall and Goldsmith, 1983; Harvey and Bedrak, 1984).

Proudman and Corcoran (1981) and Corcoran and Proudman (1991) have demonstrated that there are three different isoforms of turkey PRL, each having different binding affinities for PRL receptors. They have reported that nonglycosylated trPRLs are more easily isolated than the glycosylated forms. It is possible that some existing trPRL radioimmunoassays use the nonglycosylated forms and some may use the glycosylated forms. Turkey and chicken PRL differ at only 3 of the 40 N-terminal amino acids (Proudman and Corcoran, 1981), and this raises the possibility that the chPRL radioimmunoassay used in this investigation could be used as a heterologous assay for trPRL as well. Since there is binding, perhaps nonspecific binding, of trPRL by the primary antibody in this chPRL assay, the possibility exists that the chPRL antibody may be recognizing a series of N-terminal amino acids on trPRL which are the same as those on chPRL. As long as there is a consistent difference between laying and broody turkey hens in PRL concentrations, it would be possible to use a heterologous assay such as this chPRL radioimmunoassay described herein.

**Conclusion:** The chPRL radioimmunoassay described herein is reliable and accurate for chicken PRL and yields values which were comparable to some previously reported chicken PRL concentrations in plasma and in serum. Furthermore, use of this chPRL RIA as a heterologous assay for measurement of serum PRL in avian species as diverse as chicken, Coturnix and turkey seems feasible and can yield reliable and repeatable results, which appear to be lower but in parallel with results for the trPRL assay. The results obtained show that there are gender, age and reproductive status influences on serum prolactin concentrations. Females of the various species involved in this study appear to have the greater response to alterations in serum prolactin, as it is elevated in the females as compared with males, aging of females generally results in increased serum prolactin concentrations and broodiness coincidentally is associated with elevated serum prolactin in females of all species investigated. In males, there is an indication suggesting varietal influences on serum prolactin similar to the condition in females. In juvenile broiler chickens, the serum prolactin concentrations were not different between males and females and the stress of a coccidial infection did not cause any change in serum prolactin.

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