Science Journal of Agricultural Research & Management **Published By Science Journal Publication**
ISSN: 2276-6367
http://www.sjpub.org/sjar.html

Research Article Volume 2012. Article ID sjarm-166. 6 Pages. 2012. doi : 10.7237/sjarm/166

EFFECTS OF PREVENTIVE MEDICAL TREATMENT ON PLASMA LH CONCENTRATIONS IN GUINEA FOWL FEMALE UNDER NATURAL PHOTOPERIOD IN BURKINA FASO

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Accepted 3rd October, 2012

ABSTRACT

This study was designed to determine the effects of anti-helmintics and Newcastle disease vaccine on plasma LH concentration in female guinea fowl kept under natural photoperiod in the sub-humid region of Burkina Faso.Two groups of 30 guinea fowl female chicks from the same hatch were kept in the same brooding house. One group (T1) received no preventive medical treatment while the second (T2) was vaccinated against NCD and treated with an anti stress, anti trichomona and a dewormer.Plasma LH concentration was measured each week on 5 females of each group between 12 and 68 weeks of age. LH concentrations were similar for the 2 groups mainly because the individual variations were relatively high. Even thought peak's levels were higher in T2 group, mean values (ng/ml) were only numerically higher for T1 group, 1.19 ± 0.19 vs. 1.00 ± 0.62 before laying, 1.50 ± 0.26 vs. 1.49 ± 0.55 during laying, and 0.86 ± 0.59 vs. 0.59 ± 0.14 after the laying period. Mean value over the period of measurements were therefore only numerically higher in T, 1.16 ± 0.57 vs. 0.92 ± 0.80 ng/ml.
In conclusion, preventive medical treatment sharpened plasma LH secretion by increasing peaks levels and relatively reducing non peaks levels.

KEYWORD: guinea fowl, female, LH, laying, growth

INTRODUCTION

Several studies in chicken have shown that plasma LH is the only hormone specific to ovulation. The normal liberation of LH or the extraneous LH injection causes the liberation of the first polar injection causes the liberation of the first polar globule and the dehiscence of the ovarian follicle in a shorter time compared to the normal delay which is relatively longer (Fevold, 1943; Fraps et al., 1947; Olsen et al., 1947). It is suggested that different LH profiles relate respectively to the growing, puberty and laying phases in pullet (Sharp, 1975; Wilson et Sharp, 1975).
Contrary to chicken many studies are still needed to understand the gonadotropic hormones profiles in growing and laying guinea fowl female. The present study was designed to study the LH profile in guinea fowl females and its variation due to the effects of ordinary preventive medical treatments.

MATERIALS AND METHODS

Experimental site

This study was conducted in the city of Bobo-Dioulasso (lat. 11° 10' N et long. 4° 19' W), located in the west of Burkina Faso during the period from October 15, 1999 to April 28, 2000. The climate is of the soudanian type, characterized by a dry season from November to April and a rainy season from Mai to October.
During the last five years (1997 – 2001), average annual rain fall was 1060 ± 171, average annual temperature was 27.2 ± 0.4 °C; relative humidity was 52.8 ± 12.7%. Natural photoperiod was first decreasing from October to December then increasing from January to May (table 1)

Table I. Variation of daily photoperiod during the breeding of guinea fowls

Months	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep
Daily photoperiod (h)	11 : 52	11 : 36	11 : 29	11 : 33	11 : 35	12 : 03	12 : 22	12 : 38	12 : 46	12 : 42	12 : 28	12 : 11
Age (days)	0 -16	16 -31	31 -62	62 -93	93 -121	121- 152	152- 182	182- 213	213- 243	243- 274	274- 305	305- 335

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Housing and equipment

Two groups of 100 females of guinea fowls chicks were used for the study. Each group was maintained on litter floor in a 12 m^2 cell inside the same brooder house. Brooding heat was provided by oil lamps installed in each cell. The litter was composed of wood shaving. Ambient temperatures were measured with mercury thermometers and maintained at 35 ± 2°C for the first 3 weeks and at 30 ± 2°C for the last 3 weeks after which no supplementary heat was provided.

At 12 weeks of age, 30 birds in each group were wing tagged and transferred by group of 4 in 1 m2 metallic cages installed in a 15 X 6 m poultry house. Eggs were collected each day at 7H00.

Feeds

Birds were fed commercial feeds, a starter ration containing 20.3% protein and 2806 Kcal of metabolizable energy (ME) per kg during the first 12 weeks, 18.6% protein and 2700 Kcal of ME/kg until 3 months of age and then 18.6% protein and 2618 Kcal of ME/kg until the end of the experiment. Feed and water were given ad libitum at all ages.

METHODS

One group (T1) served as control and did not receive any treatment. Birds in the second group (T2) were treated:

- against stress using oxyfuran in a soluble powder form (Oxyfuran contained 40g of oxytetracycline – HCl, 80g of furaltadone – HCl, vitamins A, D_3, E, K_3, B_1, B_2, B_6, B_{12}, PP; Laprovet, France). Dosage was 1 g per liter of drinking water for 4 days.
- against trichonomas, using pills of Vermifuge Spécial Pintades (Vermifuge Spécial Pintades, VSP, contained 80mg of dimétridazole, 40mg of niclosamide, 10mg of levamisole chlorhydrate and 15 IU of vitamin A; Laprovet, France). Dosage was one pill for 0.5 kg of live weight.
- against helminths using Vermifuge Polyvalent Volailles. (Vermifuge Polyvalent Volailles, VPV, contained 160mg of niclosamide, 40mg of levamisole chlorhydrate and 60 IU of vitamin A; Laprovet, France). Dosage was one pill for 2 kg of live weight.
- against coccidioses using sodium, and 8g of diaveridine; Laprovet, France). Dosage was 1g per 4 l of drinking water.Anticox. (Anticox contained 80 g of sulfidimedine
- against New Castle disease using 0.5 ml of Ita-new vaccine per bird (Ita-new; Laprovet, France).
- Samples of faeces were collected every 2 weeks from each group for the assessment of internal parasitism. One sample of 5 birds was taken at random from each group and individually weight every week until week 68th. Each time, a blood sample was taken from each bird for plasma LH determination.

Collection of blood samples

Blood samples were collected from the wing vein trough heparinised vacuum tubes between 9h00 and 11h00. Samples were next centrifuged at 2500tr./mn for 15 mn at 4°C.

Plasma samples were separated and conserved at – 20°C until the moment of analysis.

LH dosage

LH concentrations were determined using the method described by Follet *et al.* (1972). After thawing, each plasma sample was diluted to yield 4 tubes, 2 of 40µl and 2 of 80µl. LH dilutor was added to each of these tubes to reach 200 µl.

On day 1, the 1st anti-LH antibody mixed with normal rabbit serum (NRS) is added. This mixture is added to increase the concentration of globulins to a level sufficient for immuno-precipitation.

On day 2, while tubes were incubated at 4°C for 24 h the $^{1.25}$I-LH hormone was added after 16 h of incubation. The 2nd antibody, the sheep serum which is an anti rabbit serum is added against the rabbit globulins. The reaction of immune-precipitation reaches its equilibrium after at least 24 h.

On the last day, 1 ml of diluter was added as pre-wash. The tubes were then centrifuged at 4000g for 20 mn at 4°C. The supernatant was decanted. One ml of diluter and 50 ml of water containing 5% starch in solution are added. The tubes were again centrifuged at 4000g for 15 mn at 4°C. The supernatant was discarded and the sediment was counted in a Packard counter. Results of the counter are transformed to LH concentrations using a radio-immunology software that compute the rate of decrease of $^{1.25}$I-LH in a series of tubes containing decreasing concentration of the reference hormone. Four replicates per sediment were counted.

Statistical analysis

Data collected were analysed for variance according to ANOVA procedure of SAS. Mean were separated using the LSD procedure.

RESULTS

Evolution of LH concentration with age

Concentrations of plasma LH within each group have shown little variation with age but large individual variations (table 2 and figure 1). Highest peaks were observed in T1 at the 50th week (2.02 ± 0.81 ng/ ml) and in T2 at the 30th and the 42th weeks (respectively 2.7 ± 3.2 and 2.1 ± 1.2 ng/ ml). Values of T1 were relatively higher first between the 12 and the 22th weeks and next between the 46th and the 68th weeks but relatively lower between the 30th and the 46th weeks. Lowest values were observed in T1 at the 64th and in T2 at

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the 58th week (respectively 0.68 ± 33 and 0.43 ± 0.01 ng/ ml).

Concentration values in relation with physiological states for T1 and T2 were respectively (1.12 ± 0.19 and 1.00 ± 0.62 ng/ ml) before sexual maturity, (1.50 ± 0.26 and 1.49 ± 0.551 ng/ ml) during laying and (0.86 ± 0.14 and 0.59 ± 0.14 ng/ ml) at the end of laying. No difference was found between the period means of the 2 groups. Mean values during the 56 weeks of measurements for T1 and T2 were respectively 1.16 ± 0.56 for and 0.92 0.80 ng/ ml.

Table II: Levels of plasma LH of guinea fowl females during growth and laying periods

week	12*	14	16	18	20	22	24	26	28	**30***	**32***	**34***	36	38	40	42	44	46	48	**50***	52	54	56	58	60	62	64	66	68
T1	1,1	1,0	1,0	0,8	1,3	0,9	0,9	1,1	1,3	**1,5**	**1,2**	**1,16**	1,3	1,5	1,1	1,5	1,5	1,5	1,5	**2,02**	1,6	0,9	0,7	1,09	1,0	0,9	0,68	0,7	0,9
	±	±	±	±	±	±	±	±	±	**±**	**±**	**±**	±	±	±	±	±	±	±	**±**	±	±	±	±	±	±	±	±	±
	0,2	0,3	1,1	0,2	0,7	0,3	0,1	0,1	0,2	**0,06**	**0,3**	**0,1**	0,3	0,8	0,4	1,0	0,54	0,5	0,5	**0,81**	0,9	0,02	0,4	0,5	0,4	0,4	0,33	0,4	0,4
T2	0,78	0,6	0,5	0,8	0,6	0,8	1,2	1,0	1,0	**2,65**	**2,1**	**1,84**	1,5	1,0	1,8	2,1	1,5	1,0	0,8	**0,63**	1,0	0,7	0,7	0,43	0,5	0,8	0,5	0,6	0,6
	±	±	±	±	±	±	±	±	±	**±**	**±**	**±**	±	±	±	±	±	±	±	**±**	±	±	±	±	±	±	±	±	±
	0,06	0,1	0,2	0,3	0,1	0,2	0,7	0,1	0,5	**3,2**	**0,6**	**0,7**	0,7	0,3	0,5	1,2	0,5	0,5	0,1	**0,1**	0,2	0,2	0,04	0,01	0,03	0,2	0,1	0,01	0,0

* : weeks where differences between means were statistically significant.

Relationship between plasma LH and intensity of egg laying

With T1 birds plasma LH decreased between the 30 to the 34th weeks and then increased to reach the highest level on week 30th whereas the first egg was laid 6 weeks later (figure 1). Percent eggs laid reach its maximum (14.8%) at week 40 (4 weeks later) corresponding with a decline of LH. Egg laying ceased after week 52 corresponding to the lowest level of LH. With T2 birds, plasma LH reached its highest level on week 30th but sexual maturity was set at week 32, a delay 4 weeks shorter compared to T1 (figure 2). Like in T1, maximum percent egg laid (33.8%) was reached also 4 weeks later, at week 36, corresponding also to a period of decreasing LH concentrations. Egg laying equally stopped at week 52, corresponding also to lowest levels of plasma LH. Plasma LH concentration and laying intensity have shown a strong relationship (figures 3 and 4). They were positively correlated for T1 (y = 0.06x – 0.94, r = 0.88) but negatively for T2 (y = -0.10x + 4.96, r = 0.84).

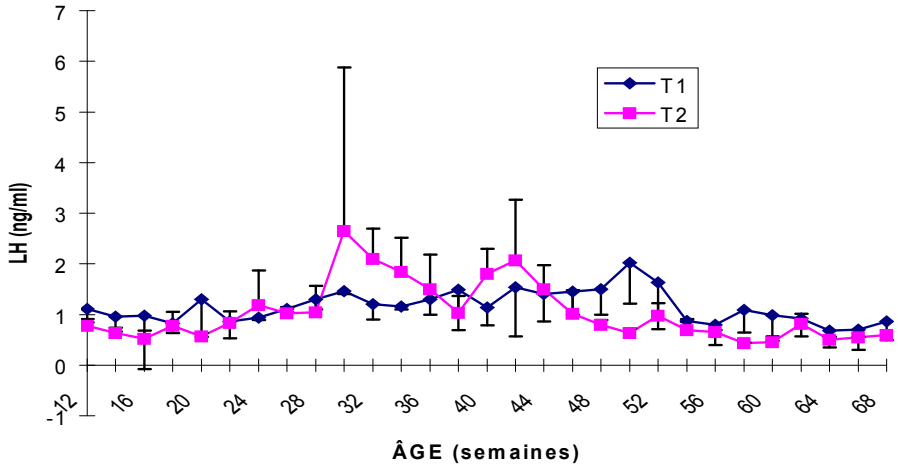

Figure 2: Weekly concentrations of LH and laying intensity of guinea fowl female of T2 group

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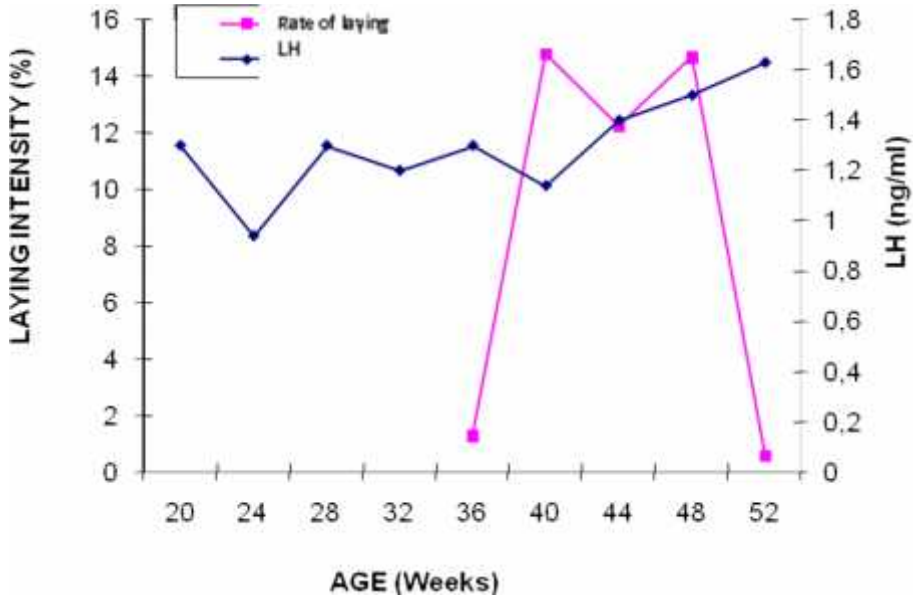

Fig. 3. Weekly LH concentrations and laying intensity in guinea fowl female of T2

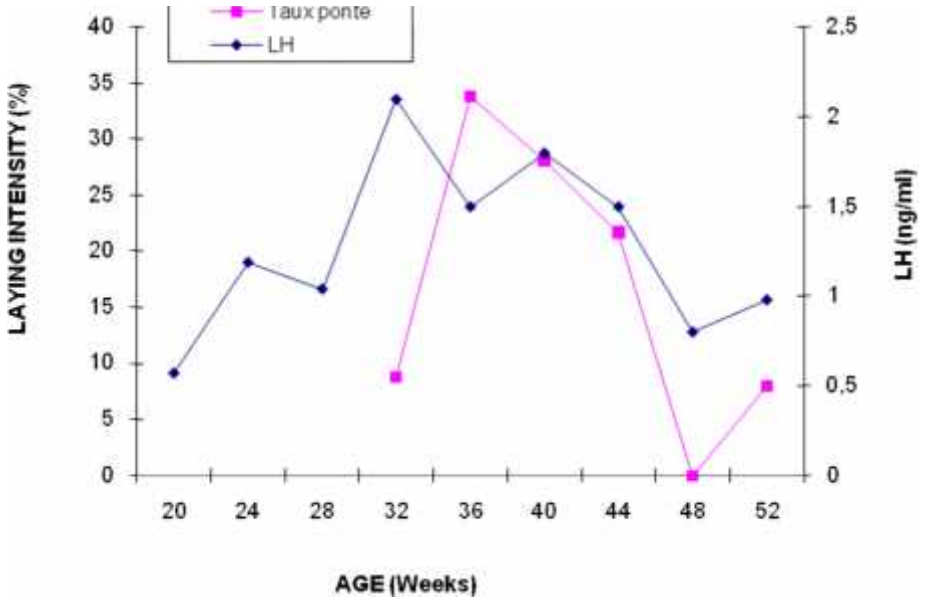

Fig. 4. Relation Relationship between Plasma LH concentrations and laying intensity in guinea fowl female of T1 group

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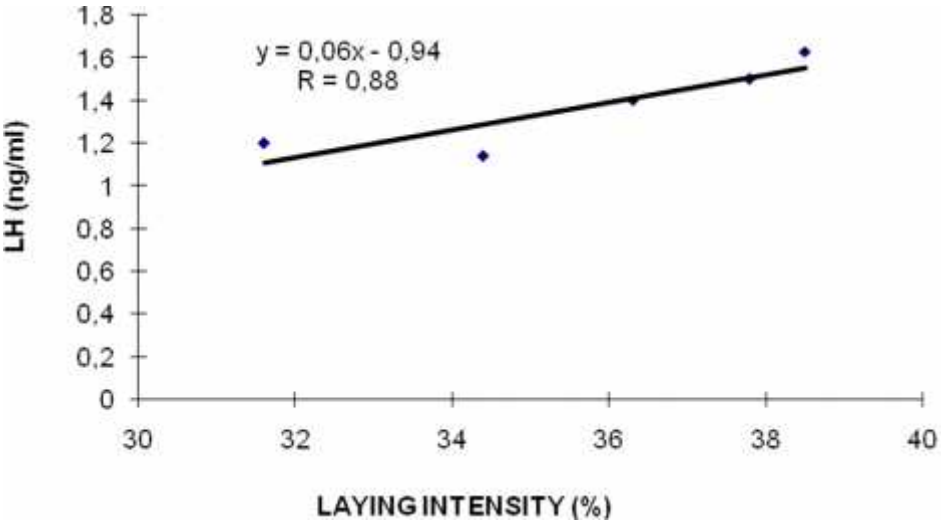
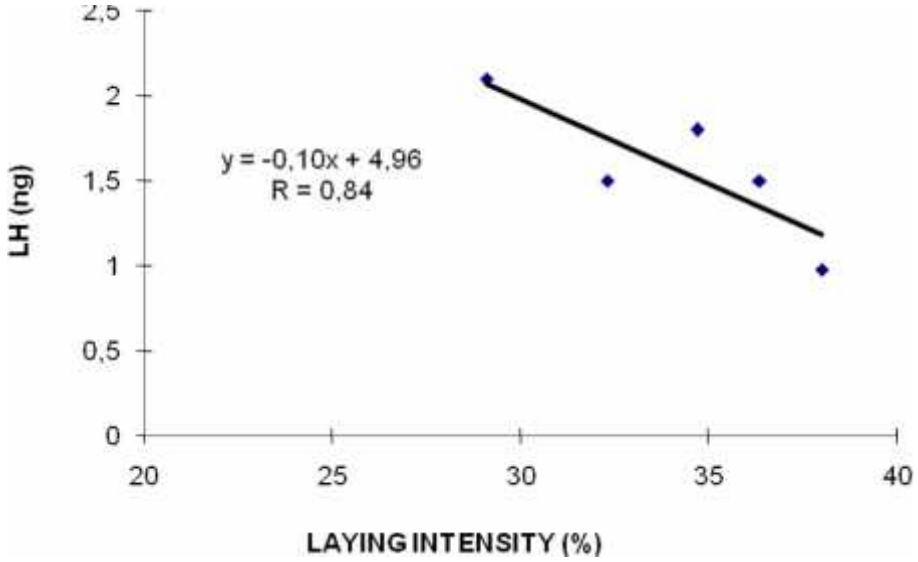


Figure 5. Relationship between Plasma LH concentrations and laying intensity in guinea fowl female of T2 group



DISCUSSIONS

Plasma LH concentrations found in this study were generally low compared to those reported for chicken. No other data for guinea fowl female is available for comparison.

The high variations observed in plasma LH concentrations between individuals of each group are characteristic of groups of animals not genetically selected. Indeed the percent egg laid and LH concentrations were not homogenous within each group.

Moreover, beside the many peaks that characterised the plasma LH curves, concentrations were found to fluctuate within a narrow range for each group. The highest variations were observed during the weeks following immediately each peak due to the shaper declines of concentrations to their lowest levels. Some of peaks appeared before puberty (pre-puberal peaks) some others after (pre-ovulatory peaks).

Variations of concentrations were also higher in T2 because amplitudes of peaks were higher in this group. A level of plasma LH as high as 7.5 ng /ml was found in one female at 30 weeks of age and another level of 2.8 ng/ml was found in another female at 34 weeks of age; the first ovoposition occurred 1 week and 2 days later (at 31 weeks and 2 days) in that group. In T1 plasma LH reached 2.5 ng /ml for one female and 2.1 ng/ml for another at 38 weeks of age with

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first ovoposition occurring at 255 days (36 weeks and 3 days).

LH profiles for both groups were relatively higher during the period of laying than during the periods before sexual maturity and after the cessation of egg laying. In chicken however, there is no affirmation that LH profiles are higher before sexual maturity than after (Sharp, 1975; Williams et Sharp, 1977).

A pre-puberal peak of LH occurs in pullets about 3 weeks before sexual maturity (Sharp, 1975; Williams et Sharp, 1977). According to Sauveur (1988), plasma LH levels in laying hens vary only slightly during a year. The main peak of LH secretion called pre-ovulatory peak occurs about 6 h before ovulation and about 32.5 hours before ovoposition. At the pre-ovulatory peak concentration of plasma LH can be 2 to 3 folds higher than the average level (Williams et Sharp, 1978). Similar phenomenon probably occurs in other domestic birds as seen in the 2 guinea fowl females of T2.

The decrease in LH concentration toward the cessation of egg laying is a phenomenon specific to birds. Indeed in the Gallus species, both the mature follicle and the circulating progesterone disappear after the laying period. In birds, only progesterone allows the procession of ovulation, œstradiol not been involved. Progesterone induces secretion of hypothalamic LH-RH which in turn triggers secretion of LH by hypophysis in a positive feedback phenomenon (Sauveur, 1988). Therefore, the absence of mature follicle suppresses the secretion of progesterone and also stops the mechanism of the positive feedback which triggers trough the hypothalamus the pre-ovulatory discharge of LH.

Unlike in this study where preventive medical treatment seemed to relatively depress the average plasma LH secretion, a report by Wilson (1978) rather showed an increased secretion due to preventive medical treatment, from 2.58 to 6.54 ng /ml in chicken pullets 12.5 to 17 weeks of age. However, like in our study, plasma LH of these pullets was also reported to decrease during the first 3 months of egg laying, from 4.42 to 2.72 ng /ml.

CONCLUSIONS

Preventive medical treatment sharpened plasma LH secretion by increasing peaks levels and relatively reducing non peaks levels

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