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Effects of Improving Health Status on Testicular Development of Guinea Fowl (*Numida meleagris*) Reared under Natural Photoperiod in the Sudanian Zone of Burkina Faso

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Abstract: This study concerns the effect of improving health status on testicular development of local guinea fowl reared under natural photoperiod. Two groups of 100 males of guinea fowls chicks were used for the study. One group (T1) served as control and did not received any treatment while the second group (T2) received several treatments including anti-stress, trichonemacid, anticoccidies, dewormer and vaccination against New Castle disease. Results showed no difference in body weight at 28 weeks of age between the 2 groups. However at 24 weeks of age birds in T2 had higher values for mean testicle weight (286 ± 70 versus 148 ± 34 mg). Parameters for seminiferous tubes were also higher for T2 birds: mean volume (74 ± 0.3 versus $63 \pm 6.6\%$ of testicle volume), mean diameter (126 ± 24 versus 89 ± 13 μ) and mean length (6.4 ± 0.4 versus 5.1 ± 1 mm). Improved health status also increased population numbers of spermatocytes I, rounds spermatides and interstitial cells. Differences were significant at 20 weeks of age for Sertoli cells. Spermatogenesis starts at 24 weeks of age for T2 birds but at 28 weeks for T1 birds.

Keys words: Guinea fowls, health program, testicle, puberty, sexual maturity

INTRODUCTION

An increasing consideration is given to local guinea fowl in Burkina Faso poultry production. This species reared essentially under scavenging conditions by villagers is the second provider of poultry meat after chicken. Actually very few paisans apply a health program in their system of rearing guinea fowls. Meanwhile studies on males and females of local guinea fowl in the environmental conditions of Burkina Faso are very few. Available literature on the reproductive characteristics of this species reported results that are proper of the photoperiod and temperature conditions of the climate of their respective experimental locations. Data on males concerned seasonal variations of testicle development of birds fed ad libitum (Barbier and Leroy, 1970) and the effects of daily photoperiod on testicular development and onset of spermatogenesis (Brillard, 1981; Brillard and Reviers, 1985).

The objective of this study was to determine the effects of a health program on testicular development in local guinea fowl reared in the environmental conditions of Burkina Faso.

MATERIALS AND METHODS

Experimental site: This study was conducted in the city of Bobo-Dioulasso (lat. $11^{\circ} 10'$ N et long. $4^{\circ} 19'$ 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 mm, average annual temperature was $27.2 \pm 0.4^{\circ}\text{C}$; relative humidity was $52.8 \pm 12.7\%$. Natural photoperiod was first decreasing from October to December then increasing from January to Mai (Table 1).

Housing and equipment: Two groups of 100 males 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 $35 \pm 2^{\circ}\text{C}$ for the first 3 weeks and at $30 \pm 2^{\circ}\text{C}$ for the last 3 after which no supplementary heat was provided. At 12 weeks of age, birds in each cell were wing tagged and transferred by group of 4 in 1 m^2 metallic cages installed in a bigger poultry house.

Animals: All chicks came from a single hatch of eggs artificially incubated. The eggs were bought from farms located near the experimental station. Hatchability of all eggs set was 70%. Day old chicks were not sexed.

Table 1: Average daylight hours during the period of study

Month	Average daylight (h)	Age of guinea fowls (days)
October	11.87±0.09	0-16
November	11.60±0.09	16-31
December	11.48±0.08	31-62
January	11.55±0.05	62-93
February	11.58±0.08	93-121
March	12.05±0.09	121-152
April	12.37±0.09	152-182
May	12.63±0.06	182-213

Feeds: Birds were fed commercial feeds, a starter ration containing 20.3% protein and 2806 Kcal of metabolizable energy per kg during the first 12 weeks and then a ration containing 18.6% protein and 2700 Kcal of metabolizable energy per kg until the end of the experiment. Feed and water were given *ad libitum* at all ages.

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 40 g of oxytetracycline-HCl, 80 g of furaltadone-HCl, vitamins A, D₃, E, K₃, B₁, B₂, B₆, B₁₂, PP; Laprovet, France). Dosage was 1 g per liter of drinking water for 4 days.
- Against trichomonas, using pills of Vermifuge Special Pintades (Vermifuge Special Pintades, VSP, contained 80 mg of dimetridazole, 40 mg of niclosamide, 10 mg 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 160 mg of niclosamide, 40 mg of levamisole chlorhydrate and 60 IU of vitamin A; Laprovet, France). Dosage was one pill for 2 kg of live weight.
- Against coccidiosis using Anticox. (Anticox contained 80 g of sulfidimidine sodium and 8 g of diaveridine; Laprovet, France). Dosage was 1 g per 4 l of drinking water.
- 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 on weeks 12, 14, 16, 18, 20, 22, 24, 26 and 28. Each time, the birds sampled were sacrificed and their testicles were immediately cut and weight.

Histological examinations: Samples of testicles of about 2-3 cm³ were processed according to standard operating procedures previously described for quantitative histological assessment (Ortavant, 1958; Courot, 1962; Attal and Courot, 1963; Reviere, 1971a; Reviere, 1971b; Brillard, 1981).

Fixation was made during 3 weeks in a Bouin-Hollande solution containing 10% of an aqueous solution saturated with mercury chloride.

Blocs were progressively dehydrated in ethanol using a series of increasing ethanol concentration and then embedded in paraffin maintained at 57°C (Sigma, paraplast embedding media) and then cut in 7 µ thick sections using a microtone.

After removal of paraffin the obtained sections were rehydrated using a series of decreasing ethanol concentration, hydrolyzed in HCl 1 N for 17 mn at 57°C and then progressively stained with Meyer hemalum lacquer between 5 sec to 10 min. The hydrolysate was decoloured with Feulgen-Bleu Alcian (8 G X, for 9 min at ambient temperature) and mounted between slides and cover glasses for optical microscope observation.

Necessary measurements for the quantification of germinal and somatic cells production were made using a drawing tube and a graphic table. Measured parameters were relative volume (Vr), diameter and total length of seminiferous tubes.

The Vr, the volume of testicle occupied by the seminiferous tubes was determined using a technique previously described (Brown *et al.*, 1975), tested in cockerels (Reviere, 1971a), in guinea fowl (Brillard, 1981) and (Brillard and Reviere, 1985) and in turkey tom (Noirault, 1999). Through this method, 28 microscopic fields chosen at random on testicle transversal sections are observed (magnification 200 x) on a 25 - points ocular (Henning grill, Olympus optical CO, Tokyo, Japan).

The number of points situated on the seminiferous tubes (tube membranes included) is used to estimate the percentage of testicle occupied by the tubes. The difference (100-% tubes) is the percentage of intertubular mass of the gonad.

$$Vr \text{ is computed as } Vr = P/\rho \times \% \text{ tubes}$$

Where:

P = Testicle weight

ρ = 1.05, specific weight of testicle tissue (Reviere, 1971a)

Histological contraction "C" is the fraction (fresh volume of testicle-testicle volume/fresh volume of testicle x 100) (Attal and Courot, 1963).

The average value obtained for C is 33.4% (coefficient of variation: 13.1%), measured with a spirometer on 10 testicles weighing between 100-800 mg.

The average diameter (Ø) of seminiferous tubes was obtained by measuring 2 orthogonal diameters with a graduated ocular. For the measure of tube section (S), observations were made (at magnification 250 x) only on tubes which minimum and maximum diameters differ by less than 15%. On each testicular section, measurements were made on 10 transversal sections of seminiferous tubes.

Table 2: Relative volume (Vr), average diameter (\emptyset , μ) and total length (Lt) of seminiferous tubes of testicles of local guinea fowls
Age of guinea fowls (weeks)

		12	14	16	18	20	22	24	26	28
T1	Vr	46±0a	46±3b	53±2.3c	58±3.2d	59±3.5e	59±2.2f	63±6.6g	66±7.5h	69±6.6i
	\emptyset	48±0a	50±3.1b	55.4±3c	60±2.6d	67±12e	86±11f	89±13g	94±11h	94±11i
	Lt	0.9±0a	1.1±0.4b	2.3±0.7c	3.1±0.7d	3.3±1e	4.3±1f	5.1±1g	5.5±1.4h	6.1±1.3i
T2	Vr	46±0a	47±2b	56.3±4c	58±5d	59±3.5e	60±1.2f	74±0.3j	82±6.3k	84±1.3l
	\emptyset	48±0a	49±1.7b	58.7±4c	60±5.2d	67±12e	90±16f	126±24j	171±31k	215±13l
	Lt	0.9±0a	1.1±0.4b	2.7±0.7c	3.5±1d	4.6±1.2e	3.9±0.6f	6.4±0.4j	7.4±1h	8.1±1.3k

Means in the same column bearing different letters are significantly different ($p < 0.05$)

Table 3: Weekly numbers of Spermatocytes I (SI), Round Spermatids (SR), SR/SI fraction, base cells and Sertoli cells (BcSc) and Interstitial Cells (IC) by transversal section of seminiferous tubes

		14	16	18	20	22	24	26	28	
T1	SI ($\times 10^6$)	Nt/2test	0.2±0.1	0.3±0.02	1±0.6	1±0.4	3±1.2	4±3.9	7±7	10±19
	SR ($\times 10^6$)	Nt/2test	0	0	0	0	0	0	0	0.1
	SR/SI (1)	Nt/2test	0	0	0	0	0	0	0	0.01
	BcSc ($\times 10^6$)	Nt/2test	3±1	4±1	8±3	10±3	24±7	26±15	31±20	40±
	IC ($\times 10^6$)	Nt/2test		-	9±2	-	11±2	13±3	15±2	18±3
	SI ($\times 10^6$)	Nt/2test	0.3±0	1±0.6	1.1±0.4	2.4±1.2	2.4±0.1	24±8	45±17	79±13
T2	SR ($\times 10^6$)	Nt/2test	0	0	0	0	0	3±2	26±2	106±37
	SR/ SI	Nt/2test	0	0	0	0	0	0.12	0.6	1.3
	CsS ($\times 10^6$)	Nt/2test	3.4±0	8±3	11±1	22±9	22±1	39±6	41±5	49±4
	CI ($\times 10^6$)	Nt/2test	0	8±1	8±1.4	9±1.5	9±1	51±3	53±3	58±4

Each mean represents the data obtained from 5 guinea fowls. Nt/2test represents the total number of cells contained in both testicles. Each mean is the average of the 2 testicles of the same bird. (1) is the meiotic fraction. Since 1 primary spermatocyte generates 4 round spermatids and their life spans are respectively 4.5 and 2.5 days, then the maximum theoretic meiotic fraction, $R_m = 4 \times 2.5/4.5 = 2.22$

Total length of seminiferous tubes (Lt) was computed as:

$$Lt = Vr \times (100-C)/S$$

Where:

$$S = \pi \times \emptyset^2/4$$

$Lt = P \times \%tubes \times (100-33.4) \times 4/\rho \times \pi \times \emptyset^2$, where units are expressed in meter (m) for Lt, gram (g) for P, micrometer (μ m) for \emptyset and g/cm^3 for ρ

Cellular counts on seminiferous tubes sections:

Spermatocytes I (without pre-leptotenes) and round spermatids have been counted on 10 transversal sections for each testicle. Counts have been made using the number of nucleus for a cell type (N_o), the thickness of a section (e) and the average diameter of these nuclei (d).

The real number of cell centers (N_c) has been computed as $N_c = N_o \times e/(e + d)$ (Abercrombie, 1946) and the total number (Nt) as $N_t = N_c \times Lt \times 10^3/e$. This procedure is more precise for spermatocytes I and round spermatids because they have nuclei with spherical shape and is less precise for base and Sertoli cells of bulls rams and cocks because these cells nuclei have shapes that are not quite spherical. It has been applied here for guinea fowl because shapes of these nuclei are considered spherical enough.

Inasmuch as the diameter can vary with testicular development, measurements on nuclear surfaces have

been made with a planimeter (ASTM LEITZ); however, while counting is easier with round spermatids, it is more difficult with spermatocytes I which can be confounded with secondary spermatocytes and therefore can induce and overestimation this category of cells.

The total number of interstitial cells (Nt int) can be estimated from the total volume of the interstitial zone (V), the average volume of a Leydig nucleus (v) and the relative frequency of this category of cells (Fr):

$$Nt \text{ int} = V \times Fr/v$$

The total volume of the interstitial zone (V) is determined from the weight of the testicle, the % of seminiferous tubes and the specific weight of testicle tissue using the formula:

$$V = \text{testicle weight} \times (100-\% \text{ tubes})/\rho$$

The average volume of an interstitial cell nucleus (v) considered spherical can be computed from the average diameter of this nucleus (average of 10 nucleus per testicle) as: $v = \pi \times \emptyset^3/6$. The relative Frequency (Fr) is measured with a network of Henning (number of points situated on the nuclei/number of points on the total interstitial zone; 21 fields per testicle). Thin and long

nucleuses have not been considered in the counts as suggested in counting for duck (Benoit, 1935a; Courot, 1971; Marchand, 1972) and guinea fowl (Barbier and Leroy, 1970).

Daily production of spermatocytes I and spermatids:

The total number of a cell population "Nt" expresses the state of development of that population at the time of measurement. It is proportional to the life span of the type of cells studied.

Duration of spermatogenesis and its different steps has been found stable in ram (Ortavant, 1956), man (Garnier, 1972), bulls (Attal and Courot, 1963), swine (Ortavant *et al.*, 1962), cocks (Reviere, 1968) and duck (Marchand *et al.*, 1977).

This particularity allows the estimation of daily production of spermatozoa or its precursors (Amann and Almquist, 1962; Kennelly and Foote, 1964; Orgebin-Crist, 1968; Reviere, 1971a; Reviere, 1971b) and also allows the estimation of meiotic yields: if "G" represents the life span of a given cell, daily testicular production "φ" of this category will equal: $\phi = Nt/G$. It will be then possible to define a meiotic ratio Rm such as $Rm = \phi \text{ spd} / R / \phi \text{ spc} / C1$, which is itself the product of the two partial yields of meiosis steps: $Rm = Rpm \times Rdm$, where Rpm is the yield of meiotic prophase (spC1 dipl./SpC1 prelept.) and Rdm the yield of meiotic division (spd/SpC1 dipl.).

Statistical analyses: Data collected were submitted to analysis of variance according to ANOVA procedure of SAS and student test.

RESULTS

Body and testicle development

Body development: Bi-weekly body weights of guinea fowl from 1-28 weeks were not different ($p > 0.05$) between the two groups (Fig. 1). The two growth curves had comparable general shapes and show that body development is composed of three different phases for each group. Average daily gain was lower (2.0 to 2.1 g for T1 and T2) during phase 1, from 1 to 8 weeks, higher (10.0 to 10.5 g) in phase 2, from 8 to 20 weeks and intermediate (4.1 to 3.8 g) in phase 3, from 20 to 28 weeks.

At 28 weeks of age, mean live weight was 918.8 ± 23.3 g for T1 and 937 ± 61.4 g for T2.

Testicle development: Testicle weight was similar between the two groups and was low (8.3 mg) during the first 12 weeks after which daily growth rates of T2 group were consistently higher ($p < 0.05$), 2.14 vs. 0.93 mg between weeks 12 and 20 and 10.9 vs. 2.4 mg between weeks 20 and 28 (Fig. 2). Mean weight at 28 weeks was therefore higher in T2, 738 ± 107 vs. 196 ± 59 mg.

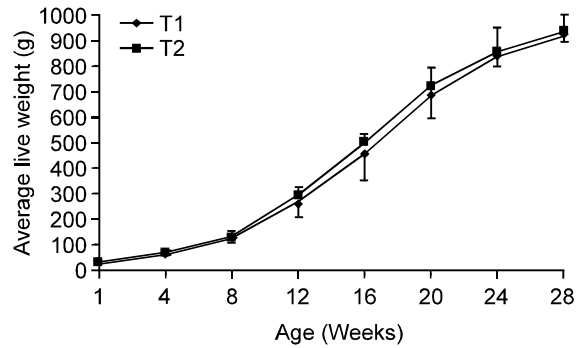


Fig. 1: Weekly average body weight (each point is the mean of data obtained on 5 guinea fowls)

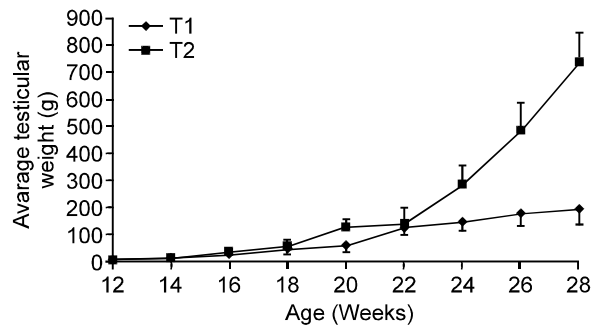


Fig. 2: Weekly testicle weight (each point is the mean of data obtained on 5 guinea fowls)

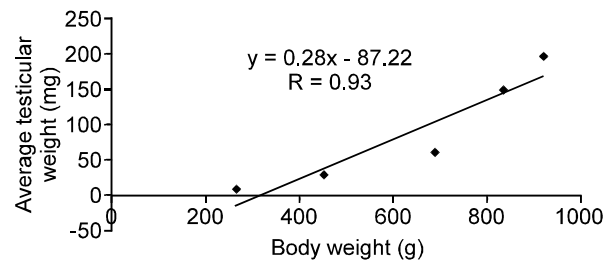


Fig. 3: Relation between testicle weight and body weight for T1 guinea fowls. Each point is the mean of data obtained on 5 guinea fowls and each data is the average of the 2 testicles of the same bird

Testicle weight and body weight were correlated linearly and similarly for the two groups during the first 18 weeks (720 g of body weight) after which positive quadratic responses were observed, being not significant in T1 but much stronger in T2 (Fig. 3 and 4). Linear correlation coefficients between weights of testicle and body were 93% in T1 but 81% in T2 because of the significant quadratic response induced by the effect of the treatments.

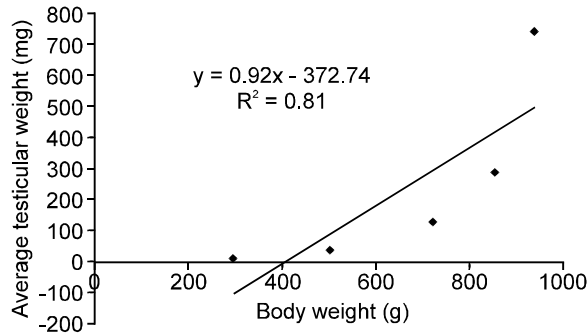


Fig. 4: Relation between testicle weight and body weight for T2 guinea fowls. Each point is the mean of data obtained on 5 guinea fowls and each data is the average of the 2 testicles of the same bird

Histological study

Relative volume of seminiferous tubes: Seminiferous tubes volume evolved similarly for both groups until week 16th, starting from 46% at week 12th to more than half the volume of the testicle content (54%) at week 16th. Strong linear relationships existed between tubes volume and testicle weight ($y = 48.5 + 0.1x$, $r = 0.92$ for T1 vs. $y = 52 + 0.1x$, $r = 0.93$ for T2; where $y = \% \text{ volume}$ and $x = \text{testicle weight}$). Higher values ($p < 0.05$), observed in T2 starting from week 24th are therefore due to higher constant because slopes are equal.

Diameter of seminiferous tube: Average tubes diameters started at 48 μm for both groups at week 12th, increased linearly but more rapidly in T2 starting from week 24th to reach 215 \pm 13 vs. 94 \pm 11 μm in T1 at week 28th. Linear equations were $y = 48.3 + 0.3x$, $r = 0.98$ for T1 and $y = 48.3 + 0.24x$, $r = 0.99$, where $y = \text{average diameter}$ and $x = \text{average testicle weight}$.

Total length of seminiferous tubes: At week 12th average length of tubes was 0.9 m for both groups after which different ($p < 0.05$) linear increases were observed to reach 6.1 \pm 1.3 m for T1 and 8.1 \pm 1.3 m for T2 at week 28th. Differences were significant starting from the 24th week of age. Total length had a strong correlation with testicle weight ($y = -1.24 + 0.008x$, $r = 0.96$, $p < 0.01$ for T1 and $y = -2.6 + 0.01x$, $r = 0.99$, $p < 0.01$ for T2, where $y = \text{average length}$ and $x = \text{average testicle weight}$).

Germinal cell numbers: No germinal cell was visible in both groups until 14 weeks of age after which spermatocytes were observed increasing in number with age. However round spermatids were observed at 28 weeks in T1 but at 24 weeks in T2 where they increased rapidly in number from 2.8 \pm 2 at 24 weeks to 106 \pm 87 millions at 28 weeks. Consequently meiotic yield in T1 was almost null until the age of 28 weeks

(0.01) and differed significantly from that of T2 between weeks 24th (0.12) and (1.3) 28th.

Base and sertoli cells: Only base cells were observed between the walls of seminiferous tubes until week 14th. A great deal of these cells migrated between the spermatocytes I starting from week 22th in T1 but 24th in T2. This migration was accompanied by important nucleus transformations that led to consider them as Sertoli cells. Average number of these cells increased considerably from 10 \pm 3 to 24 \pm 7 million between weeks 20 and 22th in T1 but from 11 \pm 1 to 22 \pm 9 million between weeks 18 and 20th in T2. The rate of increase was lessened until week 28th. Correlation between numbers of spermatocytes I and numbers of Sertoli cells was also stronger in T2 ($y = -17.18 + 1.5x$, $r = 0.88$) than in T1 ($y = -1.18 + 0.25x$, $r = 0.96$; where $y = \text{total number of spermatocytes I}$ and $x = \text{total number of Sertoli cells}$). Starting from week 24th correlation between round spermatids and Sertoli cells was significant in T2 only ($y = -394.2 + 10.2x$, $r = 0.999$; where $y = \text{total number of round spermatids}$ and $x = \text{total number of Sertoli cells}$).

Interstitial cells: Interstitial cells were observed only from week 16th for both groups. Numbers have increased slowly to reach 18 \pm 3 million (2 testicles) in week 28th for T1 but more rapidly from 51 \pm 3 millions in week 24 to 58 \pm 4 millions in week 28th for T2.

DISCUSSION

Results showed that no improving effect on testicle parameters was observed by improved sanitary condition before the 22th week, period during which individual data contained high variations.

Even though body weights were similar at 24 weeks between the 2 groups, heavier testicles in T2 can lead to believe that birds in this group had matured earlier and that body weight cannot be used to predict testicle weight (Brillard and de Reviers, 1985). Testicle growth was in phase with body growth during the first 24 weeks after which faster growth rate was observed in T2 leading to weaker linear correlation with body weight for this group, $r^2 = 81\%$ compared to 93% for T1. Another explanation of this observed variation can result from the disparity between the genetic of individual birds because the local breed has not undergone any artificial genetic selection.

Delays of growth in weight and in diameter and length of seminiferous tubes of testicle observed in T1 birds could result from the lack of medical prophylaxes which also explained the higher rate of mortality of this group (43 vs. 21% for T2). Coprology examinations revealed that coccidiosis were more responsible for the observed mortality rates. It had been shown that coccidiosis were present in all types of poultry houses and cause high mortality and decreased performances in infected birds (Chalkley, 1943; Courrot, 1962).

Comparatively, the 62 mg recorded for the 2 testicles weight at 20 weeks in this study were much smaller than the 1572 mg reported by Brillard (Brillard and Reviere, 1981) for a breed that has undergone genetic selection. Also at this age the volume of testicle occupied by seminiferous tubes was 59% in our study but 90% in Brillard's study (Brillard and Reviere, 1981).

Similarly diameter and length of seminiferous tubes were also smaller. The same author (Benoit, 1935a) had explained that environmental adaptation and genetic selection can induce fundamental modifications in morphologic characteristics and in production performances such as sexual precocity in guinea fowl.

On the other end, as in Brillard's study (Brillard and Reviere, 1981), our study also found that testicle growth rate was closely correlated with testicle parameters: volume, diameter and length of seminiferous tubes.

Puberty was reached 4 weeks earlier due to improved medical conditions (T2), manifested by earlier apparition of long spermatids, spermatozoa, interstitial and Sertoli cells and round spermatids.

At the onset of puberty, the meiotic fraction is 1.3, which was reached by T2 group at 28 weeks of age which was also 16 weeks late than the age of 12 weeks reported by Brillard's study (Brillard and Reviere, 1981).

However, onset of sexual maturity is an event not as sharp in the male as is the lay of the 1st egg in the female (Reviere, 1971a) because the relation of the age of onset and the quality of reproductive life is a question in the male.

Studies on males of chicken (Ortavant, 1958; Ortavant *et al.*, 1962; Reviere, 1968) and guinea fowl (Benoit, 1935b; Brillard, 1981; Brillard and Reviere, 1981) have shown that for both species puberty can be induced earlier by submitting males to increased hours of day light and that earliness of puberty induces lower testicle weight at the end of growing period because during the growing period, testicle weight increases proportionally to the length of the delay observed before day light hours are increased. In other words, testicle weight increases proportionally to the length of the growing period (Ortavant *et al.*, 1962).

Consequently guinea fowls reared in traditional condition with no improvement in health, feed and environmental status should have a much longer growing period and thus heavier testicle weight.

The apparent antagonism between sexual precocity and adult testicle weight seem to be linked to factors involved in the multiplication process of Sertoli cells (Brillard, 1981). The gonadotropic hormones, LH and FSH are in this sense implicated in the control of these cell populations before puberty (Brillard and Reviere, 1981; Garnier, 1972). The reason why this antagonism exists is not well known, but could consist of a retro action problem from the testicular steroids (Ortavant *et al.*, 1962).

Conclusion: Improving medical status induced early puberty and sexual maturity in local guinea fowls reared under natural photoperiod in the traditional system of Burkina Faso, but not as much as in those reared constantly under 14 h of day light.

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