

# A COMPARISON OF PRODUCTIVITY TRAITS OF DAMS, SHEDDERS AND NON-SHEDDERS OF THE GROUP-SPECIFIC ANTIGEN OF AVIAN LYMPHOID LEUKOSIS VIRUSES AND THEIR PROGENY<sup>1</sup>

CARLOS H. ROMERO<sup>2</sup>, CHERYL A. ROWE<sup>2</sup>, PAULO GENARO O. DIAS<sup>3</sup>, OSVALDO A. RESENDE<sup>3</sup>, MARIA WANDA SANTOS<sup>3</sup> E JOANNA M.L. MONTEIRO<sup>3</sup>

**SINOPSE.**- Romero C.H., Rowe C.A., Dias P.G.O., Resende O.A., Santos M.W. & Monteiro J.M.L. 1983. [Comparação nos parâmetros de produtividade de matrizes, eliminadoras e não eliminadoras do antígeno específico de grupo dos vírus da leucose linfóide aviária e de sua progênie.] A comparison of productivity traits of dams, shedders and non-shedders of the group-specific antigen of avian lymphoid leukosis viruses and their progeny. *Pesquisa Veterinária Brasileira* 3(1):11-16. Embrapa - Patologia Animal, Km 47, Seropédica, RJ 23460, Brazil.

Matrizes Leghorn brancas de duas linhagens que tinham sido selecionadas como não eliminadoras do antígeno específico de grupo (ag-gs) dos vírus da leucose linfóide (VLL) aviária na albumina de ovos frescos não incubados, produziram um maior número de ovos ( $p < 0,01$ ) durante sete períodos consecutivos de postura de 28 dias cada um, que as matrizes das mesmas linhagens selecionadas como eliminadoras constantes de ag-gs dos VLL aviária. Ovos foram obtidos de todas as matrizes, incubados e vários parâmetros de produtividade foram comparados. Os ovos obtidos das matrizes não eliminadoras de ag-gs tiveram maior fertilidade ( $p < 0,01$ ) que os ovos obtidos de matrizes eliminadoras da mesma linhagem. Uma melhor viabilidade embrionária foi somente observada em uma das linhagens não eliminadoras de ag-gs. Os ovos férteis derivados das duas linhagens não eliminadoras tiveram uma eclosão superior ( $p < 0,01$ ) e percentagem maior de pintos eclodidos de boa qualidade ( $p < 0,01$ ) que os ovos das matrizes eliminadoras correspondentes. As progênies obtidas das linhagens não eliminadoras acusaram menor mortalidade ( $p < 0,01$ ) e maior ganho de peso corporal ( $p < 0,01$ ) que as progênies obtidas das linhagens eliminadoras. Conclui-se que a infecção inaparente de matrizes Leghorn brancas com os VLL aviária afeta tanto a produção de ovos, como a fertilidade, viabilidade e eclodibilidade dos mesmos, assim como o crescimento e a mortalidade observada durante as primeiras semanas de vida. Desde que, a metodologia disponível para reduzir as taxas de infecção ou erradicar os VLL aviária é relativamente simples, recomenda-se que as firmas fornecedoras de aves matrizes operando no Brasil, iniciem programas de controle dos VLL aviária nos seus plantéis de avós.

**TERMOS DE INDEXAÇÃO:** Leucose linfóide aviária, vírus, produtividade, erradicação.

**ABSTRACT.**- White Leghorn hens of two lines, selected as non-shedders of the group-specific antigen (gs-ag) of avian lymphoid leukosis viruses (LLV) in the albumen of their fresh unincubated eggs, produced significantly more eggs ( $p < 0.01$ ) during seven consecutive laying periods of 28 days each, than hens of the same lines that had been selected as consistent shedders of the gs-ag of LLV. Eggs were obtained from all dams, incubated, and various parameters of productivity compared. Eggs obtained from non-shedder dams had better fertility ( $p < 0.01$ ) than eggs obtained from shedder dams of the same line. Superior embryo viability was observed in only one of the non-shedder lines. Fertile eggs of both

non-shedder lines had better hatchability ( $p < 0.01$ ) and yielded a higher percentage of good quality chicks ( $p < 0.01$ ) than eggs from correspondig shedder hens. Progeny obtained from non-shedder dams had lower non-specific mortality ( $p < 0.01$ ) and a higher rate of body weight gain ( $p < 0.01$ ) than the progeny obtained from shedder dams. It is concluded that inapparent infection of White Leghorn dams with avian LLV reduces egg production and affects the productivity of their progeny. Since the methodology available for reducing infection rates or eradicating LLV is relatively simple, it is recommended that Brazilian poultry suppliers start programs to control exogenous LLV in their grandparental stocks.

**INDEX TERMS:** Avian lymphoid leukosis, virus, productivity, eradication.

<sup>1</sup> Accepted for publication on July 1, 1982.

<sup>2</sup> Unidade de Pesquisa de Patologia Animal, EMBRAPA, Seropédica, Rio de Janeiro 23460, Brazil.

<sup>3</sup> Área de Avicultura, Estação Experimental de Itaguaí, PESAGRO - Rio, Seropédica, RJ 23460, Brazil.

## INTRODUCTION

Exogenous lymphoid leukosis viruses (LLV) are ubiquitous in nature and induce tumors of various origins in susceptible

chickens (Purchase and Burmester 1978), the most common being lymphoid tumors originating in the bursa of Fabricius (Cooper et al. 1968). Flocks can be heavily infected, as evidenced by high rates of LLV-shedding into their eggs, and yet exhibit little or no tumor mortality (Crittenden and Witter 1978, Romero and Rowe unpublished results). Thus, in the absence of obvious specific morbidity or mortality, losses related to an inapparent persistent infection with LLV are difficult to estimate.

The development of simple serological techniques (Spencer et al. 1976, Romero 1977, Smith et al. 1979) has made it possible to identify dams that shed the group-specific (gs) antigen (ag) of LLV into their eggs. Inbred hens that naturally produce high levels of endogenous LLV (Crittenden et al. 1977), do not shed gs-ag into their egg albumen unless inoculated with an exogenous LLV (Romero 1977). Therefore, it can be assumed, that the direct complement fixation assay for gs-ag in the albumen of unincubated fresh eggs detects vertical transmission of exogenous, but not endogenous LLV.

The objective of the present study was to compare several productivity traits of dams and their progeny, of two lines of commercial White Leghorn chickens. Dams from these lines were selected because they consistently shed or did not shed the gs-ag of LLV into the albumen of their eggs.

## MATERIALS AND METHODS

### Chickens

All chickens were Single Comb White Leghorn of the lines A and B (Cunha Filho and Monteiro 1971) of the Área de Avicultura, Estação Experimental de Itaguaí, PESAGRO - Rio.

### Housing

Dams were reared on the floor in conventional pens until they were 16 weeks old and then transferred to individual laying cages where they were tested, and artificially inseminated to obtain progeny.

### Testing of dams

Unincubated fresh eggs obtained from all dams were tested for the presence of the gs-ag of LLV by a complement fixation test as previously described (Romero et al. 1978). Dams were selected on the basis of having produced three or six eggs, all positive (A+, B+) or negative (A-, B-) for the presence of gs-ag in the albumen. One egg was tested at the beginning of the laying period and the next two after one and two months respectively. The next three eggs were collected and tested four months apart. Daily egg production of all dams was recorded after the third testing and the results expressed as the percentage of egg production hen/day during seven laying periods of 28 days each.

### Experimental design

Two trials were performed, the first consisting of five replicate experiments and the second of three.

**Trial 1.** Ten-month-old dams that had produced three gs-ag positive or three gs-ag negative eggs were artificially inseminated twice a week. After two weeks of inseminations, eggs were collected, held at 15°C for two weeks and incubated. All eggs were candled on the seventh day to assess their fertility. Fertile eggs were candled on the 18th day to assess embryo viability. After the second candling, positive and negative eggs were separated and transferred to two identical hatching incubators. Hatched chicks were counted and the number of chicks of good quality, based on physical appearance, was determined. Chicks were wingbanded, vaccinated against Marek's disease with a commercial HVT vaccine, allocated to groups containing 50 chicks and reared in 2.5 x 2.0 m pens located in an open field. In the case of the positive progeny of both lines, group numbers were completed up to 50 by

adding negative chicks of the same line. To determine the rate of body weight gain in the positive and negative progeny of both lines, chicks were weighed on the day of hatching and individual weights were recorded every week up to the eighth week. Similarly, the mortality of the growing chicks was recorded.

**Trial 2.** Eggs for incubation were obtained from the same dams of Trial 1 after they had produced a total of either six gs-ag positive or six gs-ag negative eggs. These dams were artificially inseminated only once a week. Procedures and observations were carried out as in Trial 1.

### Statistical analysis

Data related to egg production (transformed to  $\arcsin/\%$ ) and to body weight gain of the progeny were subjected to analysis of variance and the differences determined by the Tukey's test (Snedecor 1956). Other productivity traits such as fertility, embryo liveability, hatchability, quality of one-day-old chicks and non-specific mortality up to eight weeks of age were analyzed by the chi-square test.

## DISCUSSION

The results of the present study show that inapparent infection of White Leghorn dams with LLV, as judged by the demonstration of gs-ag in the albumen of unincubated fresh eggs, adversely affects parameters of productivity such as egg production, egg fertility, viability of the embryo, hatchability, quality of the newly hatched chick, along with the weight gain and liveability of the growing chicken.

Spencer et al. (1979) showed that by selecting hens for high egg production the percentage of eggs containing gs-ag was markedly reduced. This is indirect evidence to support our conclusion that by eliminating dams that produce eggs containing the gs-ag of LLV in the albumen, one selects for a population of dams with increased productivity. Gavora et al. (1980) observed that shedder dams reached sexual maturity later, produced fewer and smaller eggs, and had a higher non-specific mortality than non-shedder dams. More recently, Payne et al. (1982) confirmed these findings by showing that the laying house mortality of a population selected as LLV-free was lower than that of the unselected population, and that LLV infected hens laid, on the average, 30 eggs less than LLV-free hens. The results of our work also indicate that egg production is depressed by inapparent infection with LLV, both White Leghorn lines studied being similarly affected.

Significant differences in egg fertility were observed only in trial 1, where dams had been inseminated twice a week and overall fertility in both lines was higher than in the second trial, regardless of infection with LLV. One could speculate that with lower overall fertility rates these differences would not be apparent. However, when the parameter measured was embryo liveability, no significant differences were observed in trial 1, whereas in trial 2, embryos from non-shedder dams of line A had a higher percentage of viability than those from shedder dams of the same line. Differences in the rate of hatching were more pronounced in line A in both trials. This finding suggests that line A is more severely affected by the infection with LLV than line B, a trend that was also apparent in the progeny of shedder dams in which non-specific mortality was higher in line A than in line B.

As a rule, groups of progeny obtained from non-shedder dams were homogeneous in size and appearance during the

## SHEDDERS AND NON-SHEDDERS OF THE GROUP-SPECIFIC ANTIGEN OF AVIAN LYMPHOID LEUKOSIS VIRUSES

Table 1. Percentage egg production hen/day in dams<sup>(a)</sup>, shedders and non-shedders of the group-specific antigen of LLV

LLV status of dams <sup>(b)</sup>	Laying period (28 days)							Mean
	1	2	3	4	5	6	7	
A+	54.1( 44) <sup>(c)</sup>	48.9	46.2	37.9	33.0	19.1	12.8	36.0
A-	67.0(144)	60.2	58.3	56.2	48.7	34.2	27.6	50.3** <sup>(d)</sup>
B+	50.8( 48)	48.8	40.7	39.0	30.6	14.8	17.5	34.6
B-	60.0(265)	59.3	60.5	58.0	46.0	30.8	25.6	48.6**

(a) Hens were 10 month old at the beginning of first laying period.

(b) Shedders (A+, B+) and non-shedders (A-, B-) of the gs-ag of LLV.

(c) Percentage egg production hen/day (number of hens).

(d) Means of egg production hen/day of positive and negative dams within lines analysed by Tukey's test.

\*\*  $p < 0.01$

first eight weeks of life. In contrast, the groups of progeny from shedder dams contained a number of stunted, dwarfed and poorly developed chickens, some of which wasted away and died: a profound anemia of undetermined origin was usually found at necropsy. Although our results on the rate of weight gain pertain to White Leghorn chickens, it is tempting to speculate that similar results might be obtained with broiler progeny from shedder dams, giving the inapparent infection with LLV unprecedented economic importance.

The mechanism by which the presence of LLV in the host depresses productivity in the absence of the tumoral form of the disease is unknown. Wainberg et al. (1980) have shown, using an *in vitro* assay considered to be a correlate of cellular immunity, that infectious and inactivated LLV, when incubated with peripheral blood leukocytes, significantly reduce their mitogenic response to ConA and PHA. Moreover, Smith and Ivanyi (1980) have shown that there is an embryonal stem cell around the time of seeding to organs that seems to act as a virus-susceptible target cell. LLV could similarly affect other types of target cells that normally mediate functions different from the immune ones, causing a depression of some of the parameters measured in the present study.

Genetic stocks from poultry suppliers operating in Brazil have been shown to be infected with LLV (Rowe et al. 1981). Rates of gs-ag shedding in the albumen ranged from 6.7% to 24.7%. The present results provide evidence that attempts to reduce rates of infection or eradicate exogenous LLV from these stocks will lead to an increase in egg production, fertility, hatchability, more good quality one-day-old chicks and less non-specific mortality during the growing period. The relatively simple direct complement fixation test and the recently developed ELISA test (Smith et al. 1979; Clark and

Dougherty 1980) could be successfully utilized to achieve these objectives, with the consequent economic benefits.

## RESULTS

The percentage of egg production hen/day of the non-shedder dams of both lines was significantly higher ( $p < 0.01$ ) than the egg production of the shedder dams over 28 weeks. The non-shedder hens of lines A and B produced 14.3% and 14.0% more eggs, respectively, than the shedder hens of the corresponding line, as measured by egg production hen/day (Table 1).

In general, eggs from dams inseminated twice a week had higher fertility rates than eggs obtained from dams that were inseminated only once a week. Since the number of inseminations could have affected the parameters under study through hatching, results from both trials are presented separately in Table 2. In trial 1, dams that shed the gs-ag of LLV in the egg albumen, consistently produced a lower percentage of fertile eggs than non-shedder dams of the same line ( $p < 0.01$ ). The average decrease was 4.3% in line A and 3.8% in line B.

Significant differences in embryo viability were only observed in trial 2, where the liveability of embryos of the non-shedder dams of line A was 14.8% better ( $p < 0.01$ ) than that of the embryos of line A shedder dams (Table 2). When the performance of viable embryos for hatching was evaluated it was found that generally, embryos derived from shedder dams died inside the shell at higher rates than embryos derived from non-shedders. Eggs from non-shedder dams of line A had better hatchability in both trials ( $p < 0.01$ ) when compared to those from shedder dams. The hatchability of eggs from

non-shedder dams of line B was significantly higher only in trial 2 ( $p < 0.05$ ). When newly hatched chicks were classified on the basis of physical appearance, it was found that eggs obtained from non-shedder dams of both lines consistently gave rise to more chicks of good quality than eggs obtained from shedder dams of the corresponding line ( $p < 0.01$ ).

Data on chicken mortality and body weight gain from the eight experiments of the two trials were pooled. The mortality recorded for the progeny of the non-shedder dams of line A was significantly lower ( $p < 0.01$ ) than the mortality of the progeny of the shedder dams of the same line (Table 3).

Likewise, the mortality of the progeny of non-shedder dams of line B was lower ( $p < 0.05$ ) than the mortality of the progeny of the corresponding shedders.

Chickens obtained from non-shedder dams of both lines gained weight at a higher rate than chickens of the same lines derived from shedder dams (Table 4). Up to the eighth week, progeny of the non-shedder dams of line A gained, on the average, 120.5 g more ( $p < 0.01$ ) than the progeny of shedder dams of the same line. The progeny of non-shedder dams of line B gained an average of 82.1 g more than the progeny of shedder dams ( $p < 0.01$ ).

Table 2. *Fertility, viability and hatchability of incubated eggs obtained from dams shedders and non-shedders of the group-specific antigen of LLV*

LLV status of dams <sup>(a)</sup>	No. of eggs incubated <sup>(b)</sup>	No. of eggs fertile (%) <sup>(c)</sup>	No. of live embryos (%) <sup>(d)</sup>	No. of chicks hatched (%)	No. of good quality chicks (%)
<i>Trial 1</i>					
A+	222	202(91.0)	197(97.5)	156(79.2)	144(64.9)
A-	926	882(95.3)**(e)	837(94.9)	734(87.7)**	706(76.2)**
B+	252	234(92.9)	220(94.0)	181(82.3)	174(69.0)
B-	547	529(96.7)**	508(96.0)	437(86.0)	426(77.9)**
<i>Trial 2</i>					
A+	300	268(89.3)	216(80.6)	143(66.2)	124(41.3)
A-	300	281(93.7)	268(95.4)**	224(83.6)**	219(73.0)**
B+	300	265(88.3)	232(87.6)	166(71.6)	154(51.3)
B-	300	271(90.3)	246(90.8)	199(80.9)*	195(65.0)**

(a) Shedders (A+, B+) and non-shedders (A-, B-) of the gs antigen of LLV.

(b) Five replicates in trial 1 and three in trial 2.

(c) Candling at seven days of incubation.

(d) Candling for embryo viability at 18 days of incubation.

(e)  $\chi^2$  test of productivity of positive and negative progeny within lines.

\*\*  $p < 0.01$

\*  $p < 0.05$

## SHEDDERS AND NON-SHEDDERS OF THE GROUP-SPECIFIC ANTIGEN OF AVIAN LYMPHOID LEUKOSIS VIRUSES

Table 3. *Mortality of progeny from dams, shedders and non-shedders of the group-specific antigen of LLV*

LLV Status of dams <sup>(a)</sup>	Weekly mortality								Total (%)
	1	2	3	4	5	6	7	8	
A+	26/238	5/212	10/207	2/197	0/195	0/195	9/195	12/186	64/238 (26.9) <sup>(b)</sup>
A-	21/388	4/367	4/363	1/359	0/358	3/358	3/358	3/355	36/388 ( 9.3) <sup>** (c)</sup>
B+	23/349	11/326	1/315	1/314	3/313	4/310	3/306	4/303	50/349 (14.3)
B-	22/385	6/363	3/357	1/354	1/353	2/352	1/350	1/349	37/385 ( 9.6) <sup>*</sup>

(a) Shedders (A+, B+) and non-shedders (A-, B-) of the gs-ag of LLV.

(b) Number of deaths/number of chickens per group (percentage).

(c)  $\chi^2$  test of total mortality of positive and negative progeny within lines.<sup>\*\*</sup>  $P < 0.01$ <sup>\*</sup>  $P < 0.05$ Table 4. *Weekly body weight gain of progeny of dams, shedders and non-shedders of the group-specific antigen of LLV*

LLV status of dams <sup>(a)</sup>	Mean of weekly body weight (g)								
	0	1	2	3	4	5	6	7	8
A+	37.3(238) <sup>(b)</sup>	50.4	78.4	120.1	176.5	236.9	303.7	374.0	463.8
A-	38.8(388)	58.1	91.0	147.9	223.4	308.0	397.7	481.7	584.3 <sup>** (c)</sup>
B+	40.0(349)	56.2	85.4	135.3	204.0	274.2	351.0	432.1	526.3
B-	40.3(385)	60.4	95.2	156.9	236.2	310.1	408.6	493.1	608.4 <sup>**</sup>

(a) Progeny of shedders (A+, B+) and non-shedders (A-, B-) of the gs-ag of LLV.

(b) Mean of body weights in eight replicate experiments (initial number of chickens).

(c) Differences in body weight at eight weeks compared within lines by Tukey's test.

<sup>\*\*</sup>  $P < 0.01$ 

**Acknowledgments.** - We would like to thank Dr. E. J. Smith of the Regional Poultry Research Laboratory in East Lansing, Michigan, for generously supplying specific antiserum against the group-specific antigen of lymphoid leukosis viruses. This work was partially supported by grant N° 2222.1689/77 awarded by the Conselho Nacional de Desenvolvimento Científico e Tecnológico, CNPq, Brazil.

## REFERENCES

- Clark D.P. & Dougherty R.M. 1980. Detection of avian oncovirus group-specific antigens by the enzyme-linked immunosorbent assay. *J. Gen. Virol.* 47:283-291.
- Cooper M.D., Payne L.N., Dent P.B., Burmester B.R. & Good R.A. 1968. Pathogenesis of avian lymphoid leukosis. 1. Histogenesis. *J. Natl Cancer Inst.* 41:373-389.
- Crittenden L.B., Motta J.V. & Smith E.J. 1977. Genetic control of RAV-O production in chickens. *Virology* 76:90-97.
- Crittenden L.B. & Witter R.L. 1978. Studies of flocks with high mortality from lymphoid leukosis. *Avian Dis.* 22:16-23.
- Cunha Filho L.A. & Monteiro J.L.M. 1971. Capacidade geral e específica de combinação e efeitos recíprocos para vários caracteres de diferentes linhagens de Leghorn branca. *Pesq. Agropec. Bras.* 6:119-130.
- Gavara J.S., Spencer J.L., Gowe K.S. & Harris D.L. 1980. Lymphoid leukosis virus infection: effects on production and mortality and consequences in selection for high egg production. *Poultry Sci.* 59:2165-2178.
- Payne L.N., Holmes A.E., Howes K., Pattison M., Pollock D.L. & Walters D.E. 1982. Further studies on the eradication and epizootiology

- of lymphoid leukosis virus infection in a commercial strain of chickens. *Avian Pathol.* 11:145-162.
- Purchase H.G. & Burmester B.R. 1978. Leukosis/sarcoma group, p. 418-468. In: Hofstad M.S., Calnek B.W., Helmboldt C.F., Reid M.W. & Yoder Jr. H.W. (ed.) *Diseases of poultry*. 7th ed. Iowa State University Press, Ames, Iowa.
- Romero C.H. 1977. The prevention of avian lymphoid leukosis tumors with the androgen analog mibolerone: pathological, virological and immunological studies. PhD dissertation, Michigan State University.
- Romero C.H., Purchase H.G., Frank F., Crittenden L.B. & Chang T.S. 1978. The prevention of natural and experimental avian lymphoid leukosis with the androgen analogue mibolerone. *Avian Pathol.* 7: 87-103.
- Rowe C.A., Romero C.H., Santos M.W., Dias P.G.O. & Resende O.A. 1981. Congenital transmission of the group-specific antigen of avian lymphoid leukosis virus in commercial stocks in Brazil. *Pesq. Vet. Bras.* 1:53-54.
- Smith E.J., Fadly A. & Okazaki W. 1979. An enzyme-linked immunosorbent assay for detecting avian leukosis-sarcoma viruses. *Avian Dis.* 23:698-707.
- Smith R.E. & Ivanyi J. 1980. Pathogenesis of virus-induced osteopetrosis in the chicken. *J. Immunol.* 125:523-530.
- Snedecor G.W. 1956. *Statistical methods*. 5th ed. Iowa State College Press, Ames, Iowa.
- Spencer J.L., Crittenden L.B., Burmester B.R., Romero C. & Witter R.L. 1976. Lymphoid leukosis viruses and gs antigen in unincubated chicken eggs. *Avian Pathol.* 5:221-226.
- Spencer J.L., Gavora J.S. & Gowe R.S. 1979. Effect of selection for high egg production in chickens on shedding of lymphoid leukosis virus and gs antigen into eggs. *Poultry Sci.* 58:279-284.
- Wainberg M.A., Beuss B. & Israel E. 1980. Virus-mediated abrogation of chicken lymphocyte responsiveness to mitogenic stimulus. *Avian Dis.* 24:580-590.