

## High Ejaculation Frequency Enhances Semen Production in Taiwan Country Chickens\*

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**ABSTRACT :** The objective of this study were to investigate the effect of ejaculation frequency on semen characteristics and to establish a method for quick assessment of sperm concentration in TCC using packed cell volume (PCV) as the parameter (Trial 1). Eighty senior roosters, averaging 61 wk-old, were used and the sperm concentrations were determined using a hemacytometer. The PCV value was measured in a capillary (0.75 mm in inner diameter) by centrifugation. A simple linear regression analysis suggested that the sperm concentrations were significantly correlated with PCV values ( $r=0.62$ ,  $p<0.001$ ). Trial 2 was conducted to determine the optimal ejaculation frequency of TCC roosters in a weekly semen collection program. The male birds were subjected to 1, 2, 3 or 6 ejaculations per week for four consecutive weeks and semen characteristics including ejaculation volume (EV, mL), sperm motility (%), PCV (%), sperm concentration (ESC,  $\times 10^9$ /mL), weekly sperm production (WSP,  $\times 10^9$ /wk) and average motile sperm numbers (AMSN,  $\times 10^9$ /ejac) were determined. Average EV was greater in the group with 3 ejac/wk than with only 1 ejac/wk in weeks 1 and 3 of the collection period. WSP increased with ejaculation frequency during the first 3 weeks of collection ( $p<0.05$ ). Sperm motility was better in the birds with 6 ejac/wk than in single ejaculation group for the first 2 wk and no significant differences were found for the last 2 wk of study. In contrast, the PCV value showed a trend of reduction for the first 2 wks in the 6 ejac/wk group. Surprisingly, no significant differences were detected in the AMSN among treatment groups. The weekly motile sperm production (WMSP) increased with ejaculation frequency. Based on our observation, PCV values could be used for a quick estimation of sperm concentration and an intensive semen collection program enhanced weekly sperm production in TCC roosters. (*Asian-Aust. J. Anim. Sci.* 2004, Vol 17, No. 7 : 924-929)

**Key Words :** Ejaculation Frequency, PCV, Sperm Concentration, Roosters

### INTRODUCTION

In Taiwan, more than 50% of the chicken markets are dominated by TCC (Taiwan Provincial Government, 1998), which is an upgraded local breed. The phenotype of TCC was a single comb, red to black feather, and black shank native breed, which is superior in meat quality, disease resistance, heat tolerance, poor fertility and other characteristics to the commercial broilers (Lee, 1998; Chao and Lee, 2001; Lin et al., 2003). To reduce broodiness, all TCC breeders are housed in individual cages and artificial insemination (AI) has been widely used for commercial production. In additions, due to demanding of good body shape, feather color, and comb size, only a few superior cockerels are used for reproduction. However, some of them could only produce very small volume of semen ( $< 0.01$  mL). In heavy commercial TCC broiler breeders, cockerels are bred twice a week as regularly practice, which is far too low compared to the light breeder layers. Some TCC farms overuse their roosters when the males are not available sufficiently. Sperm production of TCC roosters

reduces one fourth or greater at the age older than 50 wk. To find out how frequent the semen of TCC roosters, can be collected without adversely affecting their semen quality, the effects of various ejaculation frequencies on semen characteristics including ejaculation volume, sperm concentrations, and sperm motility were compared. The feasibility of estimating sperm concentration using the PCV of the ejaculates was also investigated in this study.

### MATERIALS AND METHODS

Two specific trials were performed in this study using TCC males with an average age of 61 wk (ranges from 59 to 63 wk) and an average body weight of 2.8 kg ( $\pm 0.51$  kg). These birds were randomly allocated to various semen collection frequencies and were arbitrarily ejaculated twice a week for a 2 wk pre-trial period.

#### Trial 1

Eighty TCC males were used to establish the regression equation of hemacytometric sperm count on the PCV of the semen. Semen from 20 birds in each group (1, 2, 3 or 6 ejac/wk) was collected and sperm concentration ( $10^9$ /mL) was estimated using both hemacytometer and PCV. A capillary of 75 mm in length and 1.1 mm in inner diameter was used for the PCV measurement. Semen samples were aspirated and filled up the capillary tube. Both ends of the

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**Table 1.** Semen characteristics of Taiwan Country Chickens (TCC) ejaculated at different frequencies each week

Frequency of ejaculation, ejac/wk	1	2	3	6
Ejaculation volume (EV), mL/ejac	0.57 <sup>b</sup>	0.55 <sup>b</sup>	0.72 <sup>a</sup>	0.68 <sup>a</sup>
Total ejaculation volume (TEV), mL/wk	0.57 <sup>d</sup>	1.10 <sup>c</sup>	2.18 <sup>b</sup>	4.10 <sup>a</sup>
Motility, %	54.5 <sup>b</sup>	69.9 <sup>a</sup>	73.4 <sup>a</sup>	68.1 <sup>a</sup>
PCV, %	11.5 <sup>a</sup>	9.2 <sup>a</sup>	10.5 <sup>a</sup>	6.1 <sup>b</sup>
Estimated sperm concentration (ESC), 10 <sup>9</sup> /mL	2.50 <sup>a</sup>	2.23 <sup>a</sup>	2.38 <sup>a</sup>	1.85 <sup>b</sup>
Average sperm number (ASN), 10 <sup>9</sup> /ejac	1.50 <sup>ab</sup>	1.21 <sup>c</sup>	1.68 <sup>a</sup>	1.26 <sup>bc</sup>
Weekly sperm production (WSP), 10 <sup>9</sup> /wk	1.50 <sup>c</sup>	2.41 <sup>c</sup>	5.08 <sup>b</sup>	7.53 <sup>a</sup>
Average motile sperm number (AMSN), 10 <sup>9</sup> /ejac	0.87 <sup>b</sup>	0.86 <sup>b</sup>	1.22 <sup>a</sup>	0.90 <sup>b</sup>
Weekly motile sperm number (WMSP), 10 <sup>9</sup> /wk	0.87 <sup>d</sup>	1.71 <sup>c</sup>	3.69 <sup>b</sup>	5.42 <sup>a</sup>

<sup>a, b, c, d</sup> Means in the same row without the same superscripts differ significantly ( $p < 0.05$ ).

capillary were sealed with clay and then centrifuged at  $1.336 \times g$  for 5 min (Kubota KN70, Japan). The percentages of the PCV values were recorded and correlated with hemacytometric sperm counts. A regression equation was established to determine the estimated sperm concentration (ESC) as a function of PCV values.

## Trial 2

Forty TCC males were randomly allocated to different semen collection regimes as in Trial 1. Semen characteristics including volume per ejaculation (mL), motility (%) and PCV (%) were measured. Sperm motility was estimated by visual appraisal of the percentage of normal motile sperm within the field of a  $10 \times$  objective under microscope. The size of the phallus was classified into three categories by measuring the longitudinal length of protrusion during eversion for semen collection. The three categories were small (scored 1,  $< 5$  mm), medium (scored 2, 5-10 mm in length), and large (scored 3,  $> 10$  mm in length).

The ESC was estimated by the regression equation established in Trial 1. Total sperm number per ejaculation (TSN) was calculated by multiplying ESC with ejaculation volume. Weekly sperm production (WSP) was calculated by multiplying ejaculated volume with ESC and ejaculation frequency. Average motile sperm number (AMSN) per ejaculation was obtained by multiplying sperm motility with TSN, and weekly motile sperm production (WMSP) was the product of AMS and ejaculation frequency.

## Statistical analysis

The regression equation of sperm concentrations on PCV in Trial 1 was established using Regression Procedure of SAS (1989). The treatments were regarded as independent variables in Trial 2. All other variables measured were dependent variables and each male was an experimental unit. The data in Trial 2 were analyzed by the General Linear Model (GLM) procedure of SAS (1989). Covariance analysis was performed on the variables including PCV, sperm concentration, sperm number, semen volume, motile sperm count, and total motile sperm count,

based on the data collected during pre-trial period as their covariates. The significances of the differences between treatments were tested using the LSMEANS procedure. Multivariate Analysis of Variance (MANOVA) was used for calculation of the partial correlations between the dependent variables, for instance, the correlations between phallus size and each of semen traits.

## RESULTS

In all variables measured, no significant differences were observed among the four semen collection frequencies during the pre-trial ejaculation period. Also, no significant differences were detected in the size or score of the phallus and neither in any of all the common semen characteristics among treatments during the pre-trial period.

### Regression of the PCV on the hemacytometric sperm count

A significant regression equation was established using hemacytometric sperm counts (or concentrations) and the PCV values. The intercept and regression coefficient were significant at the level of  $p < 0.0001$ . ESC can be estimated using the following equation:

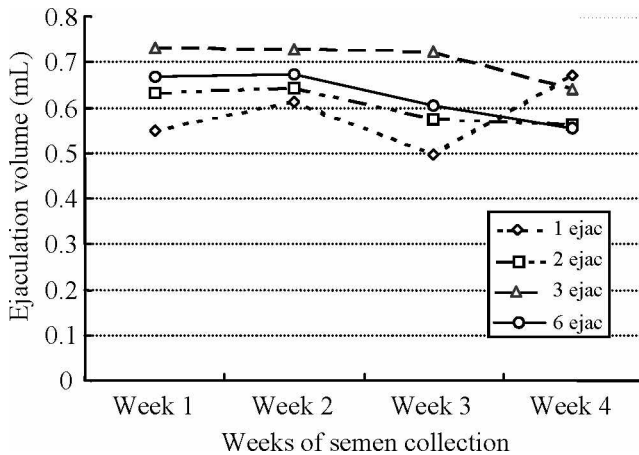
$$\text{ESC}^* (\times 10^9/\text{ejac}) = 1.095 + 0.123 \times \text{PCV} (\%)$$

Although the regression equation only expressed 39% ( $R^2 = 0.39$ ) of the variation, it was highly significant ( $p < 0.0001$ ).

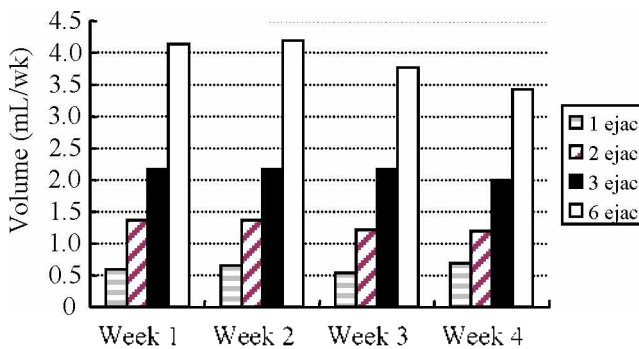
*Common semen characteristics*: All the means from the measured and calculated semen traits were pooled across the 4-wk collection period within different semen collection frequencies (Table 1). All parameters observed in this study differed highly significantly among the treatments ( $p < 0.002$  to  $p < 0.0001$ ).

### Ejaculation volume

Significant differences among treatments were observed with greater semen volume collected in 3- and 6-ejac/wk groups than in 1- and 2-ejac/wk groups (0.68-0.72 vs. 0.55-



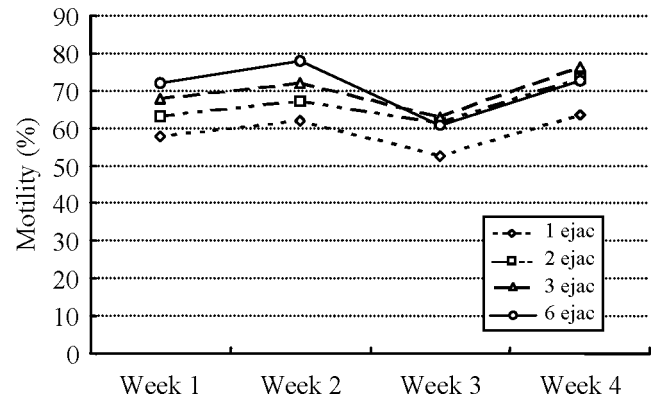
**Figure 1.** The effect of ejaculation frequency on the semen volume of Taiwan Country Chicken males during a four-week semen collection period. The X-axis represents weeks of semen collection. Means in the same week without the same letters (a, b) differ significantly ( $p < 0.05$ ). (1 ejac, 2 ejac, 3 ejac and 6 ejac represent weekly ejaculation frequencies)



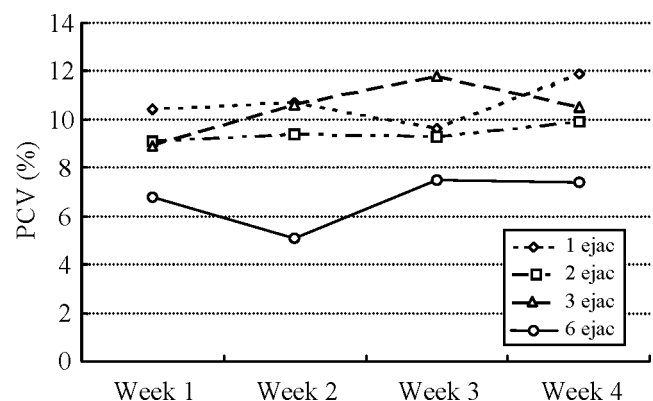
**Figure 2.** The effect of ejaculation frequency on the total ejaculation volume per week of Taiwan Country Chicken males during a four-week semen collection period. The X-axis represents weeks of semen collection. Means in the same week without the same alphabetical letters (a, b, c, d) differ significantly ( $p < 0.05$ ). (1 ejac, 2 ejac, 3 ejac and 6 ejac represent different ejaculation frequency each week)

0.57 mL,  $p < 0.05$ ; Table 1). More semen volume was obtained in the 3-ejac/wk group than the single-ejaculated birds for the first 3 weeks of collection ( $p < 0.05$ ; Figure 1). During the same period, it appeared that the birds ejaculated 3 or 6 times weekly had significant greater semen volume than those in the group subjected to only one ejaculation (0.736-0.745 and 0.663-0.738 vs. 0.48-0.59 mL). No significant difference in the semen volume was found among treatments in the last week of collection ( $p > 0.05$ ). Total ejaculation volume (TEV) increased significantly with the ejaculation frequency (Figure 2).

**Sperm motility:** A similar trend occurred in the motility of the sperm, which was better in those birds subjected to more than one ejaculation each week (68-73 vs. 55%,  $p < 0.05$ , Table 1). Male birds with 3 or 6 ejaculations each



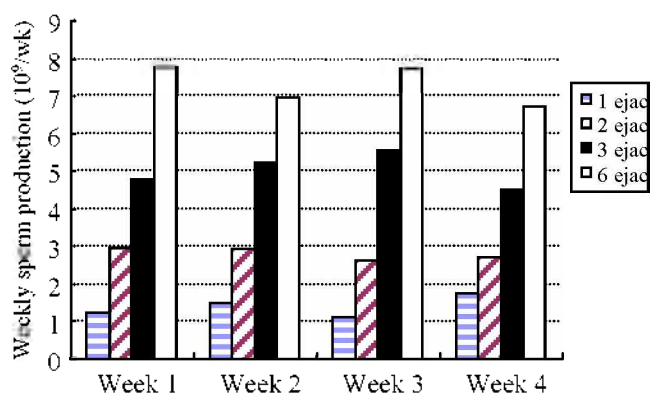
**Figure 3.** The effect of ejaculation frequency on sperm motility of Taiwan Country Chicken males during a four-week semen collection period. The X-axis represents weeks of semen collection and the Y-axis represents the percentages of motile sperm by microscopic scoring. Means in the same week without the same alphabetical letters (a, b, c) differ significantly ( $p < 0.05$ ). (1 ejac, 2 ejac, 3 ejac and 6 ejac represent different ejaculation frequencies weekly)



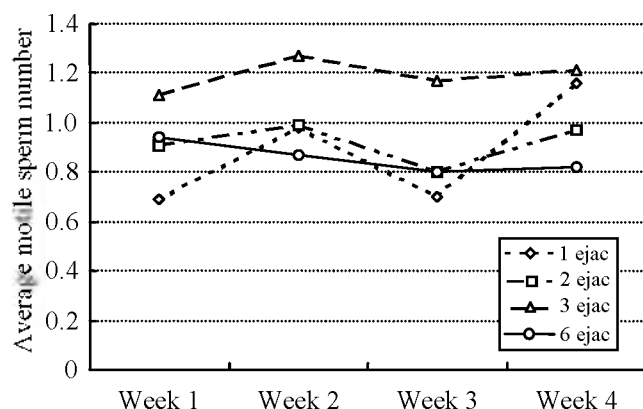
**Figure 4.** The effect of ejaculation frequency on packed cell volume (PCV) of Taiwan Country Chicken males during a four-week semen collection period. The X-axis represents weeks of semen collection. Means in the same week without the same alphabetical letters (a, b, c) differ significantly ( $p < 0.05$ ). (1 ejac, 2 ejac, 3 ejac and 6 ejac represent different ejaculation frequencies weekly)

week had better motility than those with only one ejaculation during the first two weeks of semen collection (72-75 and 69-76% vs. 53-59%,  $p < 0.05$ ), and no detectable differences were observed among treatments thereafter ( $p > 0.05$ , Figure 3).

**The PCV and estimated sperm concentrations:** In Table 1, the PCV value was significantly lower in the 6 ejac/wk group than those in other groups (1.85 vs. 2.23-2.50  $\times 10^9$ /mL) and the ESC showed exactly the same trend with the PCV value (Table 1). During the first, second and fourth weeks of semen collection, significantly reduced PCV values along the time course were observed in the birds with 6 ejaculations weekly compared to those in the single-



**Figure 5.** The effect of ejaculation frequency on weekly sperm production (WSP,  $\times 10^9/\text{wk}$ ) in Taiwan Country Chicken males during a four-week semen collection period. The X-axis represents weeks of semen collection. Means in the same week without the same alphabetical letters (a, b, c, d) differ significantly ( $p < 0.05$ ). (1 ejac, 2 ejac, 3 ejac and 6 ejac represent weekly ejaculation frequencies)

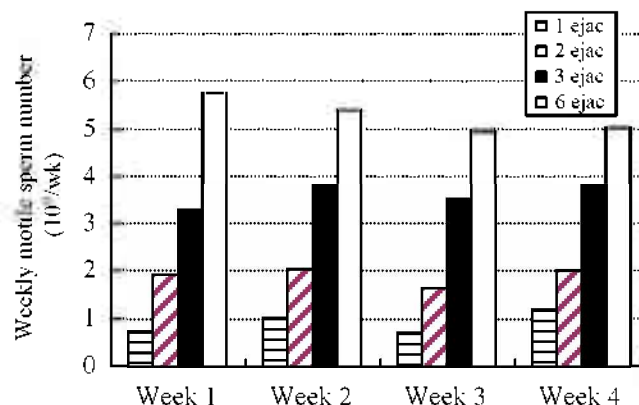


**Figure 6.** The effect of ejaculation frequency on average motile sperm number ( $\times 10^9/\text{ejac}$ ) in Taiwan Country Chicken males during a four-week semen collection period. The X-axis represents weeks of semen collection. No significant differences among groups were observed ( $p > 0.05$ ). (1 ejac, 2 ejac, 3 ejac, and 6 ejac represent different ejaculation frequencies weekly)

ejaculated group ( $p < 0.05$ , Figure 4). The ESC also maintained a similar pattern as that in PCV values (Figure 4) during the 4 wk collection period (data not shown).

**Average motile sperm and weekly sperm production :** The WSP was generally greater in the high ejaculation groups (3 or 6 ejac/wk) than in the low frequency groups (1 or 2 ejac/wk) (Table 1 and Figure 5). In Table 1, the pooled data showed that a significant greater AMSN was obtained in the 3-ejac/wk group ( $1.22 \times 10^9/\text{ejac}$ ,  $p < 0.05$ ). A pattern consistent with that occurred in WSP and WMSP increased with the collection frequency (Figure 6 and 7).

**Correlation coefficients between semen characteristics :** The partial correlation coefficients between two parameters were calculated and tested for their significance using SAS software (SAS, 1989) (Table 2). Most correlation



**Figure 7.** The effect of ejaculation frequency on weekly motile sperm production ( $\times 10^9/\text{wk}$ ) in one week in Taiwan Country Chicken males during a four-week semen collection period. The X-axis represents weeks of semen collection. Means in the same week without the same alphabetical letters (a, b, c) differ significantly ( $p < 0.05$ ). (1 ejac, 2 ejac, 3 ejac, and 6 ejac represent different ejaculation frequencies weekly)

coefficients are significant ( $p < 0.05$ ) or highly significant ( $p < 0.01$ ) except for those of PCV vs. EV ( $r = 0.07$ ), PCV vs. TEV ( $r = -0.03$ ), PCV vs. Motility ( $r = 0.02$ ), ESC vs. TEV ( $r = -0.03$ ) and ESC vs. Motility ( $r = 0.02$ ).

## DISCUSSION

The variation in animals used for experimentation has been one of the major factors influencing treatment effects. Animals at different sexual maturity might affect their reproductive performance. The birds used in this study were 59 to 63 wk of age, and no significant differences in body weight among treatment groups, which provided a uniform base line for all measurements. It is also important to have all birds in each group with a similar phallus size (score), which is exactly the case in this study.

**Prediction of sperm concentrations using PCV values :** Sperm concentrations of different animals can be directly or indirectly determined using a variety of methods (Ax et al., 2000), such as hemacytometer, automatic cell counter or optical density (Ju et al., 1985) of the diluted semen. Computer-assisted sperm analysis (CASA) is one of the most up-to-date systems for mobility assay, e.g. straight line velocity (VSL), and sperm concentration in many species. Poultry including turkey and rooster spermatozoa have been successfully analyzed using this application (Froman and Feltmann, 2000). However, it is rather costly to set up this facility. It has been known that the PCV value is positively correlated to the number of blood cells as well as the sperm in the semen of mammalian species. In attempt to establish the methodology for a quick assessment of sperm concentration, a regression equation was established to estimate ESC with a highly significant correlation

**Table 2.** Partial correlation coefficients of semen characteristics in Taiwan Country Chickens (TCC)

Frequency of ejaculation	EV <sup>1</sup>	TEV	Motility	PCV	ESC	ASN	WSP	AMSN	WMSP
Total ejaculation volume (TEV) (mL/wk)	0.85**								
Motility (%)	0.28**	0.2*							
PCV (%)	0.07	-0.03	0.02						
Estimated sperm concentration (ESC) (10 <sup>9</sup> /mL)	0.07*	-0.03	0.02	1.00**					
Average sperm number (ASN) (10 <sup>9</sup> /ejac)	0.79**	0.58**	0.19*	0.63**	0.63**				
Weekly sperm production (WSP), 10 <sup>9</sup> /wk	0.75**	0.83**	0.24**	0.37**	0.37**	0.63**			
Average motile sperm number (AMSN), 10 <sup>9</sup> /ejac	0.79**	0.62**	0.65**	0.32**	0.32**	0.80**	0.74**		
Weekly motile sperm number (WMSP), 10 <sup>9</sup> /wk	0.63**	0.74**	0.57**	0.20*	0.20*	0.58**	0.85**	0.82**	
Phallus size score (PSS)	0.50**	0.49**	0.41**	-0.16*	-0.16*	0.28**	0.40**	0.49**	0.51**

<sup>1</sup> EV: ejaculation volume, mL. \* \*\* Indicate statistical significant levels at  $p < 0.05$  and  $p < 0.01$ , respectively.

coefficient ( $r$ ) of 0.62 ( $p < 0.0001$ ,  $n = 80$ ) in Trial 1. The nature of poultry semen, which contains high proportion of particles, uric acid, and/or cloacal contaminants, may contribute to the reduced correlation coefficient and/or the precision of prediction. However, it is still relatively satisfactory for comparing sperm concentration between treatment groups.

#### Ejaculation frequency affects common sperm characteristics of TCC

*Sperm motility*: Like other species, poultry spermatozoa are immotile prior to ejaculation. Sperm acquired mobility during the transport through the ductal system and the process of epididymal maturation (Ax et al., 2000). Sperm mobility, i.e., the net movement of sperm population, is mostly determined by CASA system or Accudenz layer penetration assay. It has been shown to be a primary determinant of fertility and male fitness (Froman et al., 1999). In contrast, sperm motility is simply an expression of percentages of motile sperm under microscopic examination. Although it is essential for fertility, high motility is not necessarily indicative for fertilizing capacity of the sperm in many species (Hafez, 1993). Traditionally, sperm motility is determined by visual appraisal, by which different people might generate different results. However, to an experienced practitioner, it provides an immediate assessment of sperm viability or semen quality. This advantage is especially useful for an efficient AI practice in poultry farming.

In Table 1, sperm motility was significantly greater in the birds with 3 and 6 ejac/wk than that with only single ejaculation for the first 2 wk of semen collection (Figure 3). In weeks 3 and 4, no significant difference in sperm motility was detected. The reason is not clear, but low ejaculation frequency might result in accumulation of aged, degenerated or dead sperms in the semen, which would compromise sperm motility.

*Ejaculation volume and sperm production*: It has been noted that higher sperm concentration in avian semen ( $3\text{--}7 \times 10^9/\text{mL}$ ) compared to those in other domestic mammals ( $1.5\text{--}30 \times 10^8/\text{mL}$ ) (Garner and Hafez, 2000). The sperm

concentration for TCC roosters estimated in this study appeared in the lower range or slightly below this level (Table 1). The highest ejaculation frequency in Trial 2 (6 ejac/wk) resulted in a lower sperm production per ejaculation compared to the single ejaculated males 1.26 (1.38-1.44) vs. 1.50 (1.19-1.81)  $\times 10^9$ ,  $p < 0.05$ , Table 1). However, WSP increased 4 to 7 folds in the frequently ejaculated birds (Table 1, Figures 2 and 5) without detectable deleterious effects. Similarly, WMSP was also significantly greater in the groups with higher ejaculation frequency (3 or 6 ejac/wk), and no evident physical or physiological deteriorations were observed during the collection period (Figure 7).

In addition, approximately 3 billions of spermatozoa are produced daily by a sexually active rooster (Etches, 1996). In this study, the daily sperm production (DSP) of the TCC was theoretically close to the numbers of sperm produced by the group of 6 ejac/wk (almost 1 ejac/day). Again, it showed only half to one-third of the average DSP. This could be attributed to, at least in part, the breeding strategy of TCC production, which is preferentially limited to the selection on disease resistant, feather colors, comb shape, and meat quality. Such a strict selection of the males for maintaining the purity of strains leads to overuse of the cockerels, which could be another important cause.

Ejaculation volume and sperm concentration determine how many females can be inseminated. In Table 1, the WSPs of the roosters with 3 and 6 ejac/wk were 5.08 and  $7.53 \times 10^9$ , respectively, which implied that 50 to 75 hens could be inseminated (given  $10^8$  sperms are required for each insemination) compared to only 15 to 24 hens in the birds of 1 to 2 ejac/wk. Higher ejaculation frequency significantly increased production of semen for insemination of more hens. However, only a 4 wk semen collection period was conducted in this study. It is possible that an adverse effect could occur when the TCC roosters were tested for a longer durations, which required further investigation.

In an exhaustive ejaculation test, a sexually matured and experienced rooster being isolated for 36 h can ejaculate as many as 3-4 times within 15 min, although most of the

sperm produced is contained in the first ejaculation. The sperm concentrations range from 2.57 to  $3.17 \times 10^9$ /mL for the first ejaculation and decrease to 1.23 to  $1.96 \times 10^9$ /mL for the rest of ejaculates. Semen volume also reduced from 0.38 to 0.46 mL to 0.22 to 0.30 mL (Parker et al., 1942). It appeared that EV and sperm concentration decreased as the frequency of ejaculation increased. Interestingly, it was not the case of TCC roosters in this study. The EVs were greater in the birds with 3 or 6 ejac/wk than with only 1 to 2 ejaculations (Table 1). Although the reason is not clear, frequent stimulations to the birds via semen collection might be one of the main causes to this phenomenon.

*The effect of the phallus size on sperm production* : DSP in animal and poultry is usually positively correlated with the testicular size (de Reviere and Williams, 1984). Due to no testicular size available in this study, the phallus type become the second best indicator for male bird reproductive performance in TCC (Lee et al., 1999). Therefore, we scored the phallus of these male birds based on their sizes during ejaculation. In the analysis of partial correlation coefficients (r) of the phallus size score (PSS) with other semen traits, we found significant to highly significant correlations between PSS and all the semen characteristics measured, including EV, TEV, sperm motility, PCV, ESC, average sperm number per ejaculation (ASN), WSP, AMSN and WMSP (Table 2). Therefore, we conclude that the ability of semen production in TCC roosters may be screened by the size of the phallus in the field without measuring the DSP of the testicles, which is technically infeasible for regular AI practice in the farm.

To our knowledge, this study provided the first detailed analysis of the semen characteristics in TCC roosters. Four weeks with high frequency of semen collection in these birds enhances total sperm and motile sperm production in a week. It appears that the TCC roosters, even in the age of 61 wk, subjected to 3 and 6 ejac/wk for 4 wk showed no deterioration in spermatogenesis. In contrast, the male could be ejaculated up to 6 times per week to achieve the maximal offspring produced per TCC rooster. Further studies are required to determine whether a longer duration than 4 wk of high ejaculation frequency sustains maximal semen production.

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