Enhancement of sperm motility and viability by turmeric by-product dietary supplementation in roosters

Running title: Effects of turmeric by-product on rooster sperm

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ABSTRACT

Improving sperm motility and viability are major goals to improve efficiency in the poultry industry. In this study, the effects of supplemental dietary turmeric by-product (TBP) from commercial turmeric production on sperm motility, viability, and antioxidative status were examined in domestic fowl. Mature Rhode Island Red roosters were divided into two groups - controls (group C) without TBP administration and test subjects (group T) fed a basal diet supplemented with 0.8 g of TBP/day in a temperature-controlled rearing facility (Experiment 1) and 1.6 g/day under heat stress (Experiment 2) for 4 weeks. In Experiment 1, TBP dietary supplementation increased the sperm motility variables straight-line velocity, curvilinear velocity, and linearity based on a computer-assisted semen analysis, 2 weeks following TBP supplementation. In Experiment 2, using flow cytometry, sperm viability at 3 and 4 weeks following TBP supplementation was greater in Group T than C, and this increase was consistent with a reduction in reactive oxygen species (ROS) production at 2 and 4 weeks. The results of both experiments clearly demonstrate that dietary supplementation with TBP enhanced sperm motility in the controlled-temperature conditions as well as sperm viability, and reduced ROS generation when heat stress prevailed. Considering its potential application in a range of environments, TBP may serve as an economical and potent antioxidant to improve rooster fertility.

Keywords: Chicken; Turmeric by-product; Sperm motility; Heat stress, Reactive oxygen species

1. Introduction

For effective poultry production, sperm possessing normal motility and high viability are essential. Only motile sperm can traverse the vagina. Sperm motility is affected by physiological, nutritional, and environmental factors (Al-Qarawi, 2005). Oxidative stress also affects reproductive performance (Rui et al., 2017). High polyunsaturated fatty acids (PUFAs), sensitive to oxidative stress, in the rooster sperm plasma membrane are negatively correlated with sperm motility and viability (Surai et al., 1998). Oxidative stress may impair sperm motility (Kao et al., 2008) increase male infertility (Maya-Soriano et al., 2013; Dorostghoal et al., 2017; Li et al., 2017). Dietary supplementation with antioxidants can protect sperm from oxidative stress. For example, vitamin E protects against oxidative stress in human sperm (Keskes-Ammar et al., 2003), and zinc sulfate improves sperm motility in the Beetal goat (Rahman et al., 2014). There is need, however, for more economical and efficient antioxidant production.

Turmeric (*Curcuma longa*), a rhizomatous herb, is widely utilized as a spice, fabric dye, and herbal medicine. Turmeric by-product (TBP), resulting from commercial turmeric production, is, however, typically discarded, even though it contains curcuminoid composed of ≥80% curcumin (Ahmed and Gilani, 2014). Curcumin possesses many beneficial biological activities, e.g., anticancer, anti-inflammatory, antimicrobial, antiviral, antifungal, and antioxidant activity (Manikandan et al., 2004; Sharma et al., 2005; Aggarwal et al., 2007). Curcumin scavenges oxygen free radicals and prevent lipid peroxidation in membranes (Kuhad et al., 2007). It is, therefore, useful for the treatment of many diseases, such as cardiovascular disorders (Ramirez-Tortosa et al., 1999; Manikandan et al., 2004) and reproductive issues (Oguzturk et al., 2012; Glombik et al., 2014). Furthermore, curcumin ameliorated the harmful effects of arsenic on both the libido and semen characteristics of rabbit bucks (Seadawy et al., 2014). Effects of curcumin on sperm motility and viability in roosters are, however, unclear.

Several antioxidant substances, such as dried ginger rhizomes (Akhlaghi et al., 2014), guanidinoacetic acid (Tapeh et al., 2017), and dietary vitamin C and vitamin E (Min et al., 2016), exhibit beneficial effects on sperm motility in domestic fowl, but these results are based on visual observations and, therefore, are not objective. In other species, including pigs (Didion, 2008), cattle (Kang et al., 2015), rats (Slott et al., 1991), and humans (Liu et al., 1991), computer-assisted sperm analysis (CASA), has been applied; this method is more objective than the conventional method and provides a more comprehensive assessment of motility variables [e.g., straight-line velocity (VSL), curvilinear velocity (VCL), average path velocity (VAP), linearity (LIN, ratio of VSL/VCL), straightness (STR, VSL/VAP), amplitude of lateral head displacement (ALH) and beat-cross frequency (BCF)], which are associated with sperm fertilizability and fertility (Holt et al., 1997; Larsen et al., 2000). In addition, fluorescence-activated cell sorting analysis (FACS) is used for mammalian semen assessment, e.g., in bulls (Anzar et al., 2009) and boars (Awda et al., 2009). Assessments of rooster sperm, however, using CASA and FACS are limited (Partyka et al., 2010; Partyka et al., 2013).

In the present study, to enhance the understanding of the andrological characteristics of domestic fowl, the effectiveness of dietary TBP on rooster sperm motility and viability through the suppression of ROS generation were evaluated using the CASA system in a temperature-controlled rearing facility and using FACS when heat stress prevailed.

2. Material and methods

2.1. Turmeric by-product

Wheat meal containing 20% TBP (FL-D3575) was kindly provided by a company that processes commercial turmeric, Inabata Co.,Ltd. (Osaka, Japan). TBP, of which 4.86% (w/w) is curcuminoid according to the manufacturer, is generated during the refinery process.

2.2. Animals and sperm preparation

All experimental procedures for the care and use of animals were conducted in accordance with approved guidelines of the Animal Care Committees of Hokkaido University. The 9 to 15-month-old Rhode Island Red roosters were maintained individually imposing a photoperiod of 12-h light (L): 12-h darkness (D) (lights on at 06:00) with *ad libitum* access to water and a diet as described in Table 1. All roosters were housed separately in stainless steel cages (43.5-cm wide × 55.5-cm deep × 64.0-cm high). Semen was collected via abdominal massage using the procedure of Yamane et al. (1966). Semen obtained from each rooster was individually diluted 100-fold using Beltsville poultry semen extender (BPSE) (Sexton, 1977). The diluted semen was immediately preserved on ice after collection and then transported to the laboratory. The sperm concentrations were measured using a hemocytometer.

The diets of the control group without TBP supplementation (Group C) and the experimental group with TBP supplementation (Group T; TBP: 0.8 g/day in Experiment 1; 1.6 g/day in Experiment 2) are summarized in Figure 1 (n = 4 per group). Prior to TBP supplementation, semen was collected from each rooster and sperm were evaluated in both groups. The endpoints for sperm traits were concentrations for all experiments and motility in Experiment 1. In Experiment 2, sperm viability and oxidative status, as determined by ROS production, were evaluated in each rooster in both groups. The feed consumption rate (%) was determined weekly by measuring the

difference between provided and residual feed weights in both groups. After a 1-week acclimation period, TBP dietary supplementation was initiated for four roosters in the T group. Subsequently, semen was collected and the samples were used for each experiment at the onset of examination, which was defined as time "0" for descriptive purposes, and 2, 3, and 4 weeks after TBP supplementation initiation as shown in Figure 1. The evaluation of sperm primarily occurred 2 weeks after the initiation of TBP dietary supplementation, because rooster spermatogenesis requires 12 to 13 days from the period of meiotic prophase until the sperm pass through the lumen of the seminiferous tubules (De Reviers, 1968; Ax et al., 1976).

2.3. Motility evaluation by CASA

Sperm motility was assayed using a CASA system (SMAS, DITECT, Tokyo, Japan) coupled to a phase-contrast microscope (E200; Nikon, Tokyo, Japan). Semen samples on ice diluted with BPSE were promptly loaded onto a warmed (30 °C) 20-μm Leja 8-chamber slide (Art. No. SC20-01-08-B, Leja, Nieuw-Vennep, the Netherlands) at a final sperm concentration of 5.0×10^7 cells/ml (Ashizawa et al., 2009). The CASA system recorded 150 frames per second (fps), and sperm captured in more than 120 frames were used to calculate each parameter. To accurately assess sperm motility for each semen sample, a single microscopic field of view per chamber was examined and over 200 sperm tracks were evaluated across more than the three microscopic fields of view. Furthermore, to avoid temporal diminishment of sperm motility, each examination was completed within 30 sec of its application to the chamber. CASA software was used to evaluate the percentage of motile sperm and selectable sperm motility variables, including the motile sperm proportion (%), VSL (μm/sec), VCL (μm/sec), VAP (μm/sec), LIN (%), STR (%), ALH (μm), and BCF (Hz). Sperm with a VAP of < 5 μm/sec were excluded from analyses (Nguyen et al., 2015; Masoudi et al., 2016). In addition, according to a previous study (Froman, 2007), sperm were classified as immobile (VSL:

<30 μm/sec), or mobile (VSL: >30 μm/sec). The proportions of mobile sperm were estimated at 0, 2, 3, and 4 weeks because sperm mobility is a primary determinant of fertility in domestic fowl (Froman et al., 1999).

2.4. Flow cytometry

To detect sperm viability and oxidative status by flow cytometry, semen diluted with BPSE was mixed with three fluorescence stains as follows. First, the CellROX Deep Red Reagent fluorescent probe (Life Technologies, Grand Island, NY, USA) to evaluate ROS generation (Alves et al., 2015) was added to diluted semen samples (final concentration: 5 μM) according to the manufacturer's instructions. Subsequently, these samples were incubated for 20 min at 30 °C in the dark. After incubation, each sample was stained with SYBR-14 (final concentration 100 μM; L-7011 LIVE/DEAD Sperm Viability Kit, Molecular Probes, Eugene, OR, USA) and propidium iodide (PI) (final concentration 12 μM; L-7011, LIVE/DEAD Sperm Viability Kit, Molecular Probes) for 10 min at 30 °C.

After staining, the sperm suspensions were processed using a flow cytometer (FACS Verse; BD Biosciences, San Jose, CA, USA). SYBR-14, PI, and CellROX Deep Red were excited using a 488-nm excitation laser and detected with a FITC filter (527/32 nm), Per-CP-Cy5.5 filter (700/54 nm), and APC filter (660/10 nm), respectively. Flow cytometric gating of sperm was performed as reported previously (Nagy et al., 2003; Hallap et al., 2005; Kanno et al., 2016). Gating was performed as follows. Briefly, the particles stained with SYBR-14 and/or PI were considered to be sperm cells. Sperm were further divided into two groups (viable and nonviable) by PI emission. To assess sperm ROS generation, each group was gated according to CellROX Deep Red staining. Fluorescence data for all events were collected, and 10,000 gated events were counted. Triplicate measurements per sample were obtained and average values for individual samples were used for

analyses.

2.5. Experimental design

2.5.1. Experiment 1: Effect of TBP supplementation on sperm motility

To investigate the effect of TBP supplementation on chicken sperm motility when controlled-temperature conditions are imposed, the sperm motility was evaluated using CASA of roosters with or without TBP supplementation of the feed that was routinely used to feed the roosters. The temperature of the room was maintained at around 20 °C by air conditioning. Each rooster was housed separately. Feed consumption was measured every week and both sperm concentration and motility at 0, 2, 3, and 4 weeks were evaluated by CASA. Results were compared between Groups C and T.

2.5.2. Experiment 2: Effect of TBP supplementation on sperm viability and ROS production

To further understand the effect of TBP supplementation on rooster sperm, ROS production was examined using flow cytometry in sperm when heat stress was imposed. Generally, intracellular ROS production within an organism can be readily promoted in an open-sided housing facility without room temperature control (Ayo et al., 2011). A rearing area without air conditioning was established with the temperature conditions that were similar to the conditions of open-sided poultry houses. The examination period was in a summer month (in mid-August to early-September) and the room temperature was measured daily. As in Experiment 1, feed consumption and sperm concentration were measured in groups C and T. The feeding conditions were as shown in Table 1; the quantity of wheat meal containing TBP was doubled (1.6 g/day), because the feed consumption of each rooster was reduced by approximately half under heat stress. The oxidative status as determined by ROS production was assessed at 0, 2, 3, and 4 weeks by flow cytometry; results were

compared between groups C and T.

2.6. Statistical analysis

A statistical analysis was performed using JMP 12.0.1 (SAS, Cary, NC, USA). Data are presented as means ± SEM. The values for groups C and T were compared using Student's *t*-test. For CASA variables, sperm mobility and ROS assessment by FACS, a repeated-measures ANOVA was performed to determine whether differences were associated with TBP dietary supplementation and administration time using StatView (Abacus Concepts, Inc., Berkeley, CA, USA). Differences with $P < 0.05$ were considered significant.

3. Results

3.1. Experiment 1: TBP Supplementation enhances sperm motility

Approximately 80% of feed given to roosters was consumed in both groups C and T at 1, 2, 3, and 4 weeks (Fig. 2A). The TBP supplementation did not, therefore, appear to affect feed consumption and feeding preference of roosters. There were also no significant differences in sperm concentration between Groups at 0, 2, 3, and 4 weeks following the initiation of TBP dietary supplementation (Fig. 2B).

The effect of TBP supplementation on sperm motility was subsequently assessed by CASA. All CASA variables in Group T tended to be greater than those in Group C (Table 2). At 2 weeks after TBP supplementation, there were differences in sperm motility and VCL at 2 weeks following TBP supplementation between Groups C and T ($P < 0.05$). Additionally, VSL in Group T was greater than that in Group C at 4 weeks following TBP supplementation ($P < 0.05$). The LIN was greater in Group T than C at both 3 and 4 weeks after TBP supplementation ($P < 0.05$). The proportion of mobile sperm subpopulations as evidenced by a VSL of >30 μm/sec (Fig. 3) was greater in Group T than that C at 4 weeks after TBP supplementation ($P < 0.05$). For the CASA variables and the proportion of mobile sperm, there were no interactions of effects of TBP supplementation and administration time based on a repeated-measures ANOVA.

3.2. Experiment 2: TBP supplementation enhances sperm viability with a concomitant reduction in ROS production

To assess the effects of TBP supplementation on sperm viability and ROS production when heat stress was imposed, a rearing area without air conditioning was developed (Fig. 4A). As the experiment was performed in the summer, room temperature and humidity were both greater than during other seasons of the year and were not optimal for chicken rearing (Fig. 4B, C; Plyaschenko

and Sidorov, 1987). As expected, feed consumption in roosters decreased to 60% in both Groups C and T at 1, 2, 3, and 4 weeks (Fig. 5A); therefore, the amount of supplemented TBP was doubled (1.6 g/day). In Experiment 1, no statistical differences were detected in the sperm concentration between Groups C and T at 0, 2, 3, and 4 weeks (Fig. 5B).

By creating two-dimensional dot plots of PI and SYBR-14 fluorescence for groups C and T (Fig. 6A and B), the four subpopulations of events analyzed by flow cytometry were represented. The PI (−) SYBR (−) population was unlabeled, indicating a lack of DNA; this population was regarded as debris. The PI (−) SYBR (+) population was PI-negative but stained SYBR-14-positive, indicating that these cells had intact plasma membranes and were alive. The PI (+) SYBR (−) population represented dead cells, while the PI (+) SYBR (+) population represented dying cells. The TBP dietary supplementation led to a greater proportion of live sperm at 3 and 4 weeks than that observed for Group C ($P < 0.05$; Fig. 6C).

The ROS contents in Groups C and T were compared for the live sperm on the basis of the SYBR-14/PI analysis. In the dot plots of SYBR-14/CellROX vividly red-stained sperm, the distribution in Group T was different from that in Group C (Fig. 6D, E). The cells with a greater content of ROS were identified by CellROX vivid red fluorescence and quantified as the SYBR-14 (+) CellROX (+) population. The region of SYBR-14 (+) CellROX (+) in Group T appeared to be smaller than that in Group C. The intensity of CellROX vivid red fluorescence was compared between Groups C and T; at 2 and 4 weeks after TBP supplementation, the intensity values were less in Group T than C ($P < 0.05$; Fig. 6F). In the analysis of ROS content, there were no interactions detected between TBP supplementation and administration time based on a repeated-measures ANOVA.

4. Discussion

Effects were examined of TBP administration on rooster sperm motility, viability, and oxidative status. Curcumin, an active factor in turmeric, exhibits strong antioxidant activity comparable to that of vitamins C and E, and is, therefore, also capable of relieving oxidative stress (Maheshwari et al., 2006). Additionally, because the TBP used in the present study is basically a waste product generated during the purification of commercial turmeric, its use as a feed supplement is economically feasible for chicken feed. It was initially confirmed that TBP dietary supplementation did not prevent feed intake over a 4-week period. The intake of feed supplemented with TBP was similar to that of normal feed without TBP, even when heat stress conditions prevailed.

Results from using the CASA technologies clearly revealed that TBP dietary supplementation enhanced rooster sperm motility, consistent with the results of previous studies demonstrating beneficial effects of curcumin on sperm motility in mammals, including rats, mice, and rabbits (Oguzturk et al., 2012; Glombik et al., 2014; Seadawy et al., 2014). The CASA motility variables, [i.e., VSL (μm/sec), VCL (μm/sec), VAP (μm/sec), LIN (%), STR (%), ALH (μm), and BCF (Hz)] tended to be greater overall in Group T than C. Among these variables, there were significant differences in VSL, VCL, and LIN between groups C and T after treatment with TBP for 2 weeks. Notably, the VSL, LIN, and BCF values of freshly collected semen from turkeys was positively correlated with sperm mobility, defined as the percentage of sperm with a VSL of >30 μm/sec in roosters (Froman, 2007). The greater VSL and LIN in Group T in the current study might therefore be useful for the enhancement of sperm mobility. Hence, the dietary supplementation of TBP for at least 2 weeks clearly provided a significant benefit with respect to velocity and linearity variables, which are closely related to sperm motility in roosters.

To further clarify the effect of TBP dietary supplementation on rooster sperm, the

antioxidative status was examined of sperm when heat stress prevailed. The effects of heat stress to rooster semen dramatically decreases the sperm viability (Ayo et al., 2011) and is, therefore, detrimental to rooster fertility. In the current study, sperm viability in Group T was greater than that in Group C, and the intensity of CellROX vivid red fluorescence staining was decreased, which is indicative of cytoplasmic free radicals in ram sperm (Alves et al., 2015). Although the significant differences in sperm viability and ROS activity were not coincident at 2 and 3 weeks after TBP supplementation in Experiment 2, the significant differences in both assessments were consistent at 4 weeks (Fig. 6). This result might indicate that the stable antioxidant effect of TBP on rooster sperm required a supplementation duration of 4 weeks.

Generally, the sperm membrane is rich in PUFAs, which readily undergo lipid peroxidation by ROS, resulting in reductions in sperm motility and viability (Baumber et al., 2000). As avian sperm are characterized by relatively greater concentrations of PUFAs (Surai et al., 1998), the reductions of sperm motility and viability owing to ROS activity are greater than those in other species and are more harmful to rooster fertility. In particular, humans, mice, dogs, rabbits, and rams are 5 to 100 times more resistant than roosters to the effects of hydrogen peroxide, a representative ROS, on sperm motility (Wales et al., 1959). No studies have, however, precisely demonstrated a causal link between heat and oxidative stresses on rooster sperm viability. Results of the present study, therefore, may begin to provide a greater understanding of the means by which ROS impairs sperm properties in domestic fowl.

5. Conclusions

In conclusion, the results of the present study demonstrate that supplementation with TBP generated during the purification of commercial turmeric improves the motility and viability of rooster sperm and reduces ROS production. As active motility is essential for sperm to reach the

fertilization site in the oviduct, motility constitutes an important variable for evaluations of sperm quality in various species, including chickens. Thus, TBP dietary supplementation may serve as a useful practice for the improvement of male fertility across species. Furthermore, ambient temperature during sperm development *in vivo* is a key determinant of semen quality and fertility in roosters, particularly in countries with hot climates. The dietary supplementation of TBP may be a valuable to alleviate oxidative stress in rooster sperm during heat stress.

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Figure legends

Fig. 1. Experimental depiction summarizing roosters fed with or without supplemental TBP. Examined roosters were divided into two groups, a control group without TBP supplementation (Group C) and an experimental group with TBP supplementation (Group T) [n = 4 per group]; After a 1-week acclimation period, standard diet supplemented with TBP was provided to four roosters in the T group; Semen was collected at the onset of the examination, regarded as time "0" for descriptive purposes (0), and at 2, 3, and 4 weeks after start of TBP feeding

Fig. 2. Effects of the TBP supplementation on feed intake and sperm concentration in general room conditions. A): The rate of feed intake (%) was determined by measuring the difference between provided and residual feed weights in Groups C and T at 1, 2, 3, and 4 weeks after TBP supplementation. B): Sperm concentrations were determined using a hemocytometer at 0, 2, 3, and 4 weeks after TBP supplementation. Group C: black; group T: white (n = 4 per group)

Fig. 3. Effect of the TBP supplementation on the proportion of sperm with velocity >30 μm/sec 'Proportion of mobile sperm subpopulations showing a VSL of >30 μm/sec was calculated from the CASA results shown in Table 2' Data represented as means ± SEM; Asterisk indicates a difference between Groups C and T ($P < 0.05$)

Fig. 4. Room temperature and humidity during the experimental period when heat stress prevailed; Image of the rearing booth without air conditioning (A); As the experiment was performed in a summer season from August 1 to September 4 in Sapporo, Japan, both (B) the room temperature and (C) humidity were examined every day

Fig. 5. Effects of the TBP supplementation on feed intake and sperm concentration when heat stress prevailed A) The rate of feed intake (%) was determined by measuring the difference between provided and residual feed weights in Groups C and T at 1, 2, 3, and 4 weeks after TBP supplementation; B) Sperm concentrations were determined using a hemocytometer at 0, 2, 3, and 4 weeks after TBP supplementation; Group C: black; Group T: white (n = 4 per group)

Fig. 6. Flow cytometric detection of rooster sperm stained with SYBR-14, PI, and CellROX Deep Red; Representative dot plots of the distribution of the control sperm in Group C (A and D) and the sperm in Group T supplemented with TBP (B and E); A and B) SYBR-14, PI, and CellROX were excited using a 488-nm excitation laser and detected with an FITC filter (527/32 nm), Per-CP-Cy5.5 filter (700/54 nm), and APC filter (660/10 nm), respectively; C) The proportions (%) of live sperm in Groups C (black) and T (white) were represented at 0, 2, 3, and 4 weeks (* $P < 0.05$); D and E) Note that the region of SYBR-14 (+) CellROX (+) in Group T is smaller than that in Group C; F) The intensities of CellROX vivid red fluorescence were compared between groups C and T at 2, 3, and 4 weeks after TBP supplementation; Data in (C) and (F) are represented as means ± SEM; Asterisks indicate differences between Groups C and T ($P < 0.05$)

Table 1

Composition of the diets with or without turmeric by-product (TBP) for roosters.

	Experiment 1		Experiment 2	
Item (%)	group C	group T	group C	group T
Wheat meal	25.5	22.4	25.5	19.3
Wheat meal with turmeric by-product	0	3.1	0	6.2
Wheat bran	31.4	31.4	31.4	31.4
Corn	27.2	27.2	27.2	27.2
Oat	2.7	2.7	2.7	2.7
Soybean meal	2.7	2.7	2.7	2.7
Rice bran	0.9	0.9	0.9	0.9
Salt	0.3	0.3	0.3	0.3
Commercial formula feed	9.0	9.0	9.0	9.0
Vitamin-mineral premix	0.1	0.1	0.1	0.1

All roosters were individually maintained with the photoperiod being 12 L: 12D (lights on at 06:00) with *ad libitum* access to water and food (with or without TBP); Diets contained 0.8 and 1.6 g of TBP mixed with wheat meal in Experiments 1 and 2, respectively; Commercial formula feed (Kanematsu Agritec Co., Ltd.) was composed of corn, polished rice, milo, wheat, 56.7 g/kg; soybean cake, rapeseed meal, corn gluten meal, 12.6 g/kg; corn distillers grain soluble, 7.2 g/kg; pork meal, fish meal, 3.6 g/kg; calcium carbonate, animal fat and oil, salt, calcium phosphate, paprika extract, silicic anhydride, 9.9 g/kg; Vitamin-mineral premix was composed of vitamin A, 3,500 IU/kg; vitamin D_3, 700 IU/kg; acetic acid DL-α- tocopherol, 0.5 mg/kg; menadione sodium bisulfite, 0.4 mg/kg; thiamine nitrate, 0.5 mg/kg; riboflavin, 1 mg/kg; pyridoxine hydrochloride, 0.25mg/kg; nicotinic acid, 2 mg/kg; D-calcium pantothenate, 1 mg/kg; choline chloride, 50 mg/kg; folic acid, 0.05 mg/kg; cyanocobalamin, 0.001 mg/kg; $MnSO_4$, 32.3 mg/kg; $FeSO_4$, 16.8 mg/kg; $CoSO_4$, 0.03 mg/kg; $ZnSO_4$, 27.5 mg/kg; Gross percentage is not 100% because of truncation by setting the number of decimal places

Table 2

Effect of turmeric by-product (TBP) dietary supplementation on rooster sperm motility in controlled-temperature conditions.

	Group	0 weeks		2 weeks		3 weeks		4 weeks	
Motile Sperm (%)	C	90.14	± 1.45	84.85	± 2.41	94.51	± 1.07	92.01	± 5.22
	T	86.73	± 0.97	92.90	± 1.91*	96.67	± 0.52	95.67	± 1.88
VSL (μm/s)	C	37.65	± 4.47	24.33	± 1.08	31.45	± 2.25	26.67	± 2.38
	T	39.43	± 2.53	29.66	± 2.46	38.72	± 4.46	39.15	± 3.36*
VCL (μm/s)	C	142.94	± 6.63	101.19	± 6.22	131.07	± 3.25	121.18	± 8.21
	T	132.30	± 3.19	119.97	± 2.67*	128.01	± 11.70	135.91	± 10.01
VAP (μm/s)	C	50.98	± 5.27	36.13	± 1.93	46.07	± 1.57	39.95	± 3.04
	T	50.82	± 1.43	42.13	± 2.41	51.63	± 5.02	51.58	± 4.17
LIN (%)	C	24.92	± 2.45	24.66	± 1.20	23.75	± 1.47	22.28	± 0.89
	T	27.52	± 2.06	25.05	± 1.83	29.13	± 0.64*	28.70	± 2.03*
STR (%)	C	69.70	± 2.38	68.98	± 2.13	67.56	± 2.55	65.93	± 2.99
	T	70.76	± 3.46	70.30	± 2.30	71.38	± 1.03	73.10	± 3.04
ALH (μm)	C	2.01	± 0.13	1.71	± 0.17	1.83	± 0.12	1.78	± 0.08
	T	1.93	± 0.10	1.84	± 0.09	1.85	± 0.08	1.91	± 0.11
BCF (Hz)	C	9.83	± 0.23	10.25	± 0.84	9.14	± 0.91	9.80	± 1.03
	T	10.34	± 0.64	10.65	± 0.73	11.16	± 0.88	11.72	± 0.53

The CASA variables reflecting sperm motility were as follows: motile sperm (%), VSL (μm/sec), VCL (μm/sec), VAP (μm/sec), LIN (%), STR (%), ALH (μm), BCF (Hz); Data presented as means ± SEM; In same row, asterisks indicate differences between Groups C and T ($P < 0.05$)

9- to 18-month-old male chickens (n = 4 per group)

- Control group (C): Four roosters in the control group fed only a standard diet (▮■■■■■▮).
- Test group (T): Four roosters in the experimental group fed a diet with 0.8 g / day turmeric by-product (▬▬▬).

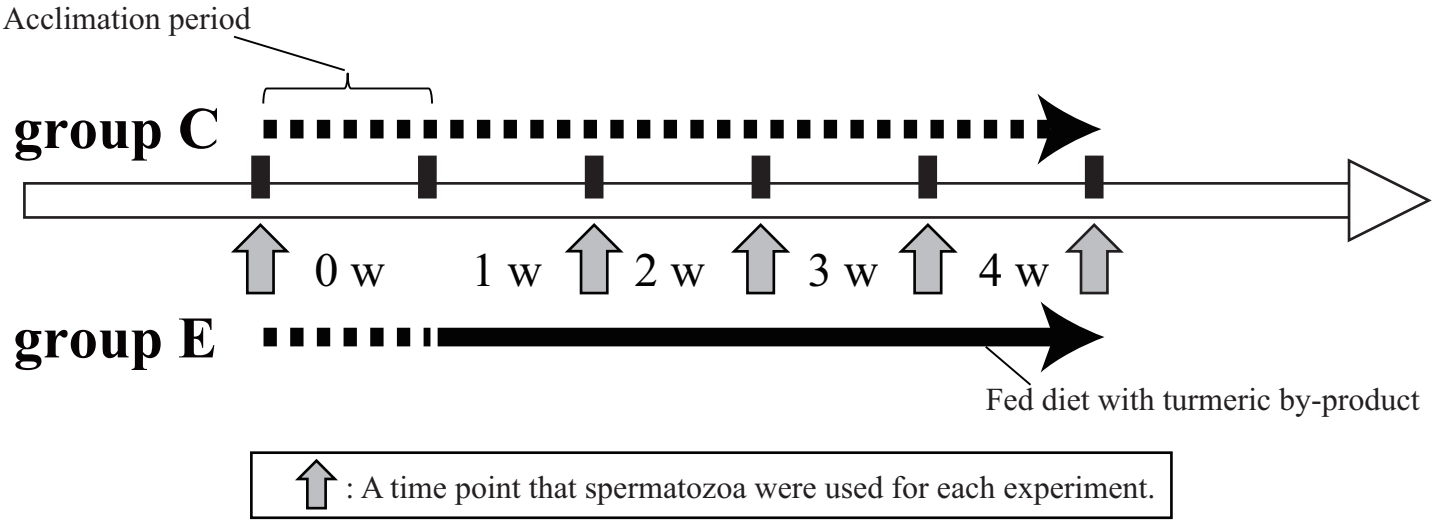
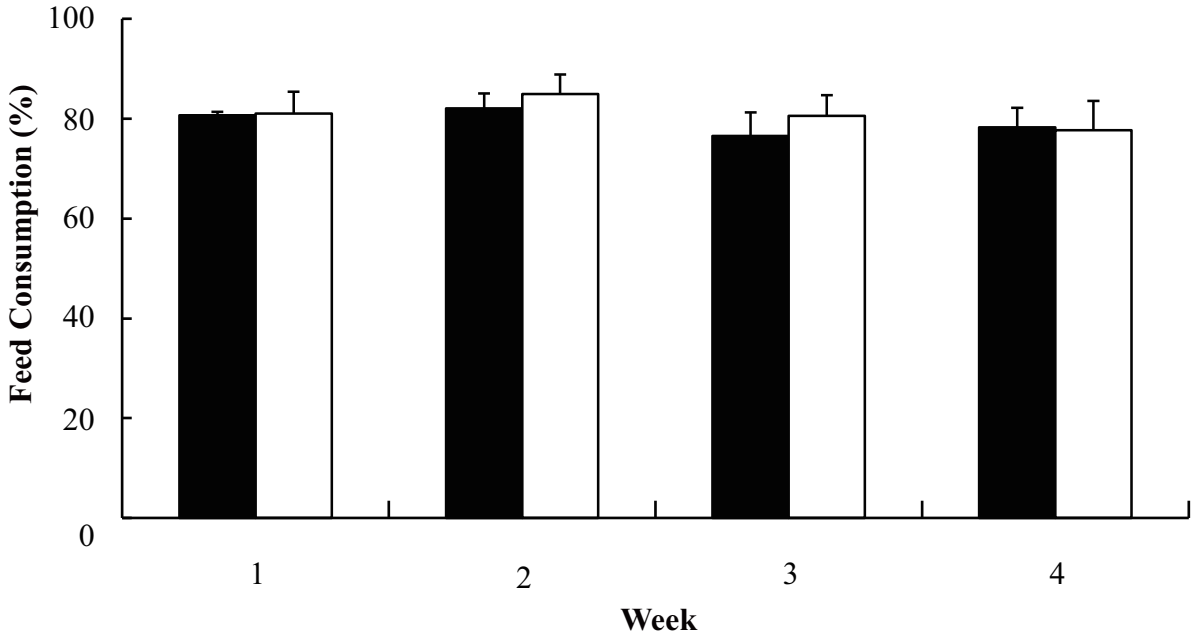


Figure 1

A


100
80
60
40
20
0
Feed Consumption (%)
1
2
3
4
Week

B

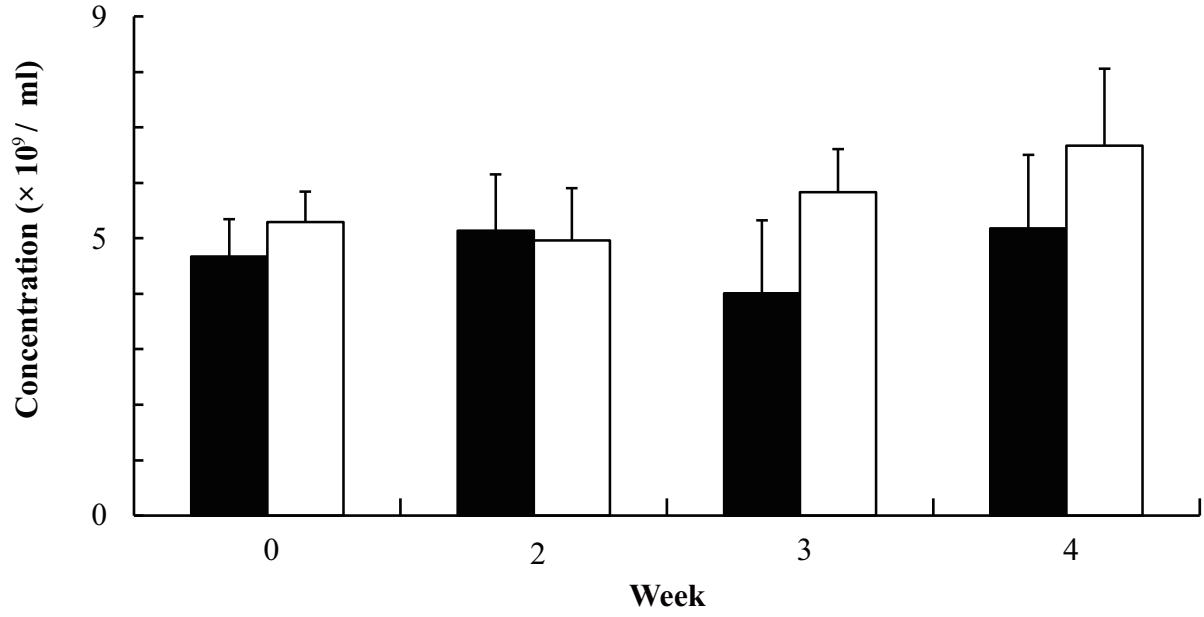

9
5
0
Concentration (× 10⁹/ ml)
0
2
3
4
Week

Figure 2

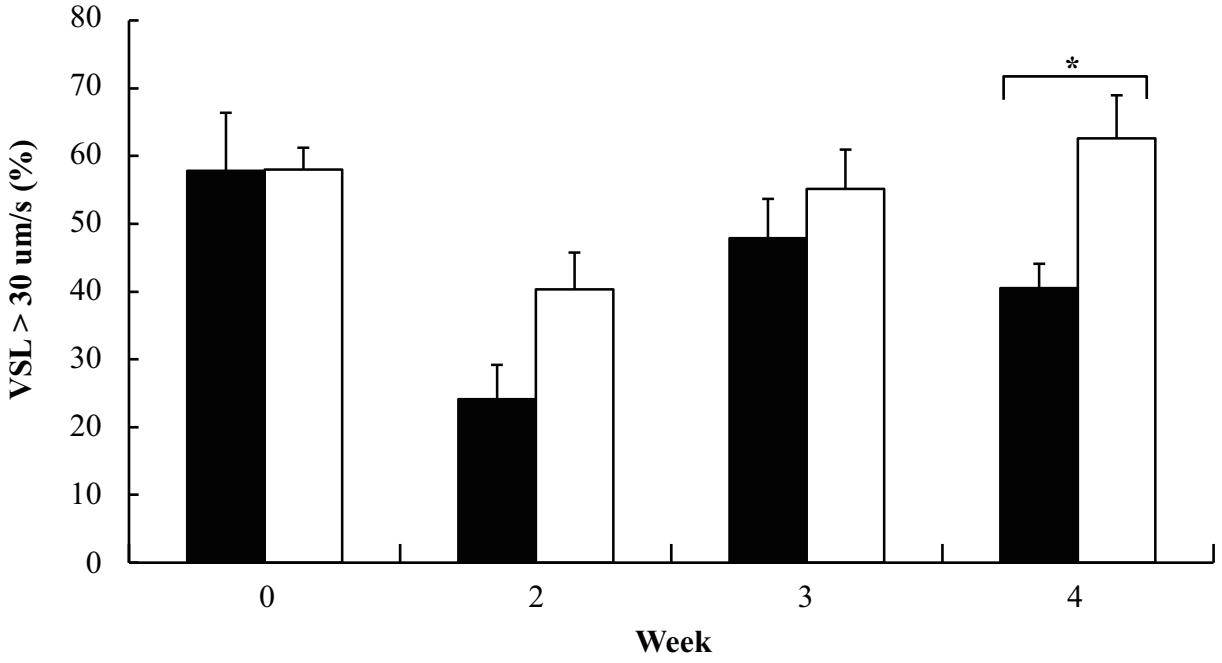
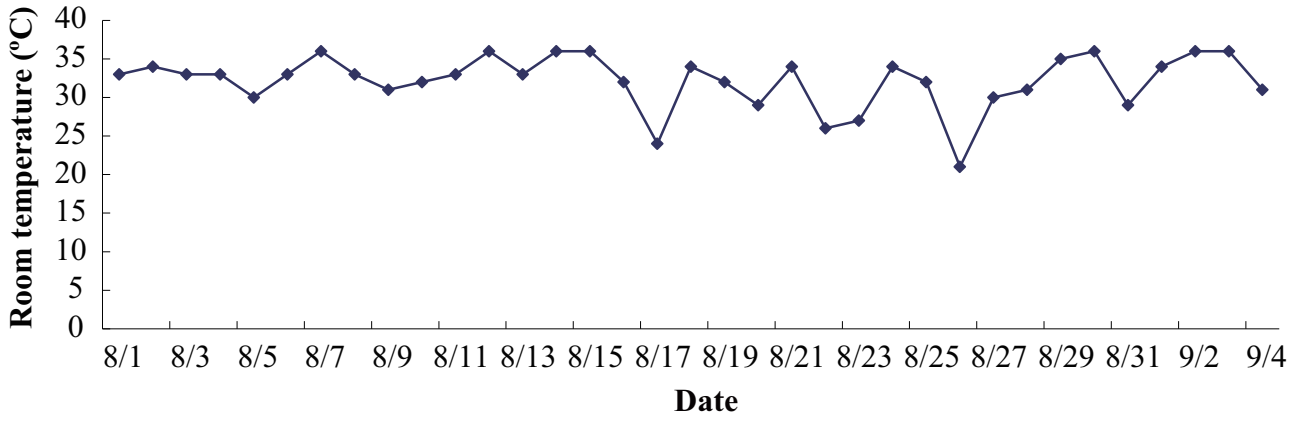


Figure 3

A

B


Room temperature (ºC)
40
35
30
25
20
15
10
5
0
8/1 8/3 8/5 8/7 8/9 8/11 8/13 8/15 8/17 8/19 8/21 8/23 8/25 8/27 8/29 8/31 9/2 9/4
Date

C

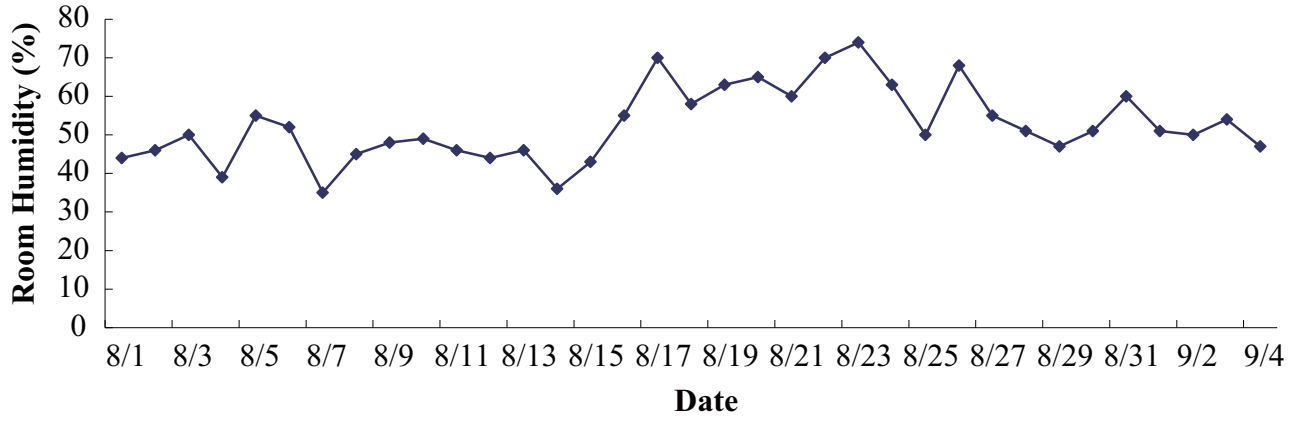
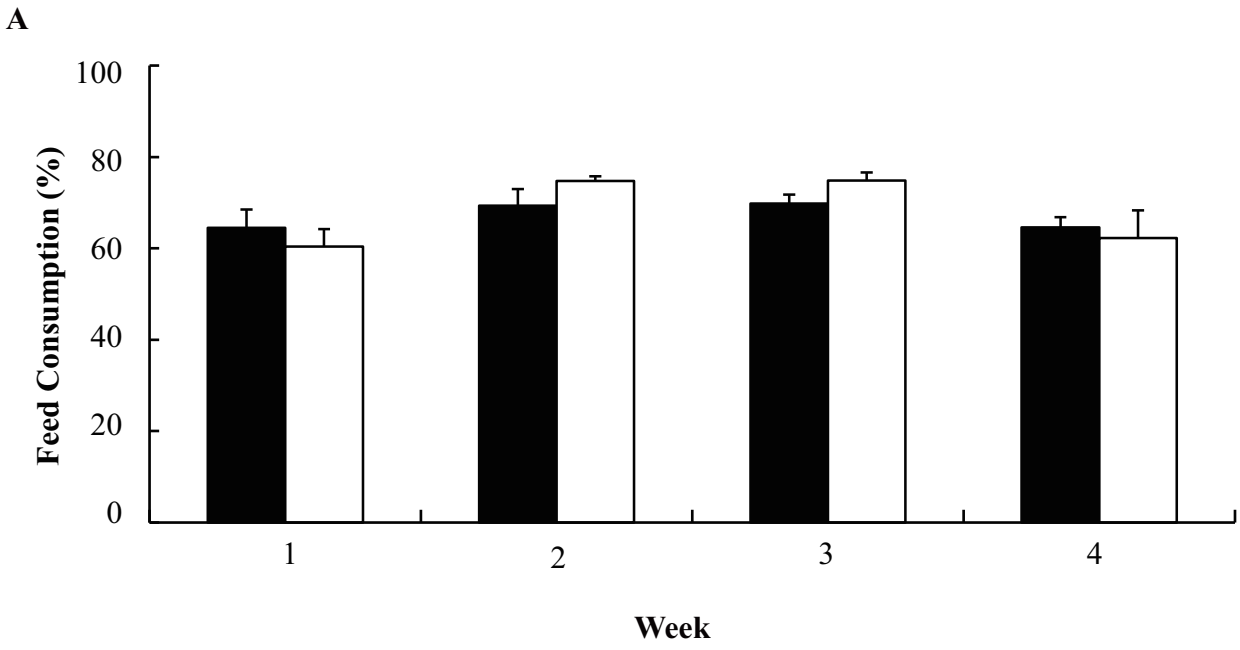

Room Humidity (%)
80
70
60
50
40
30
20
10
0
8/1 8/3 8/5 8/7 8/9 8/11 8/13 8/15 8/17 8/19 8/21 8/23 8/25 8/27 8/29 8/31 9/2 9/4
Date

Figure 4


A
100
80
60
40
20
0
Feed Consumption (%)
1
2
3
4
Week

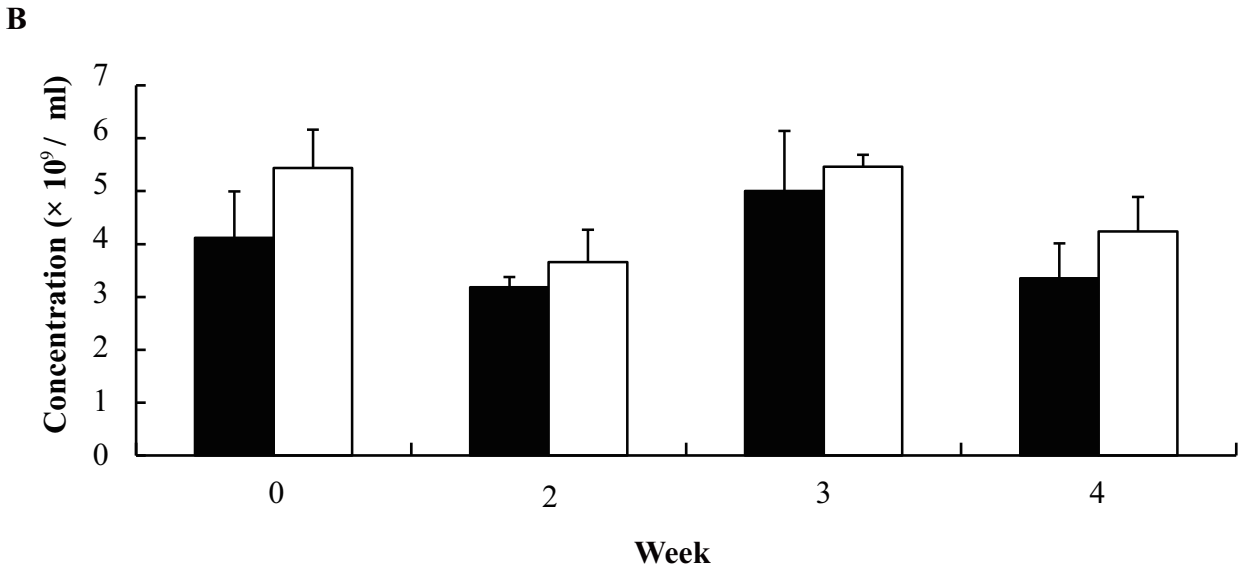

B
7
6
5
4
3
2
1
0
Concentration (× 10⁹/ ml)
0
2
3
4
Week

Figure 5

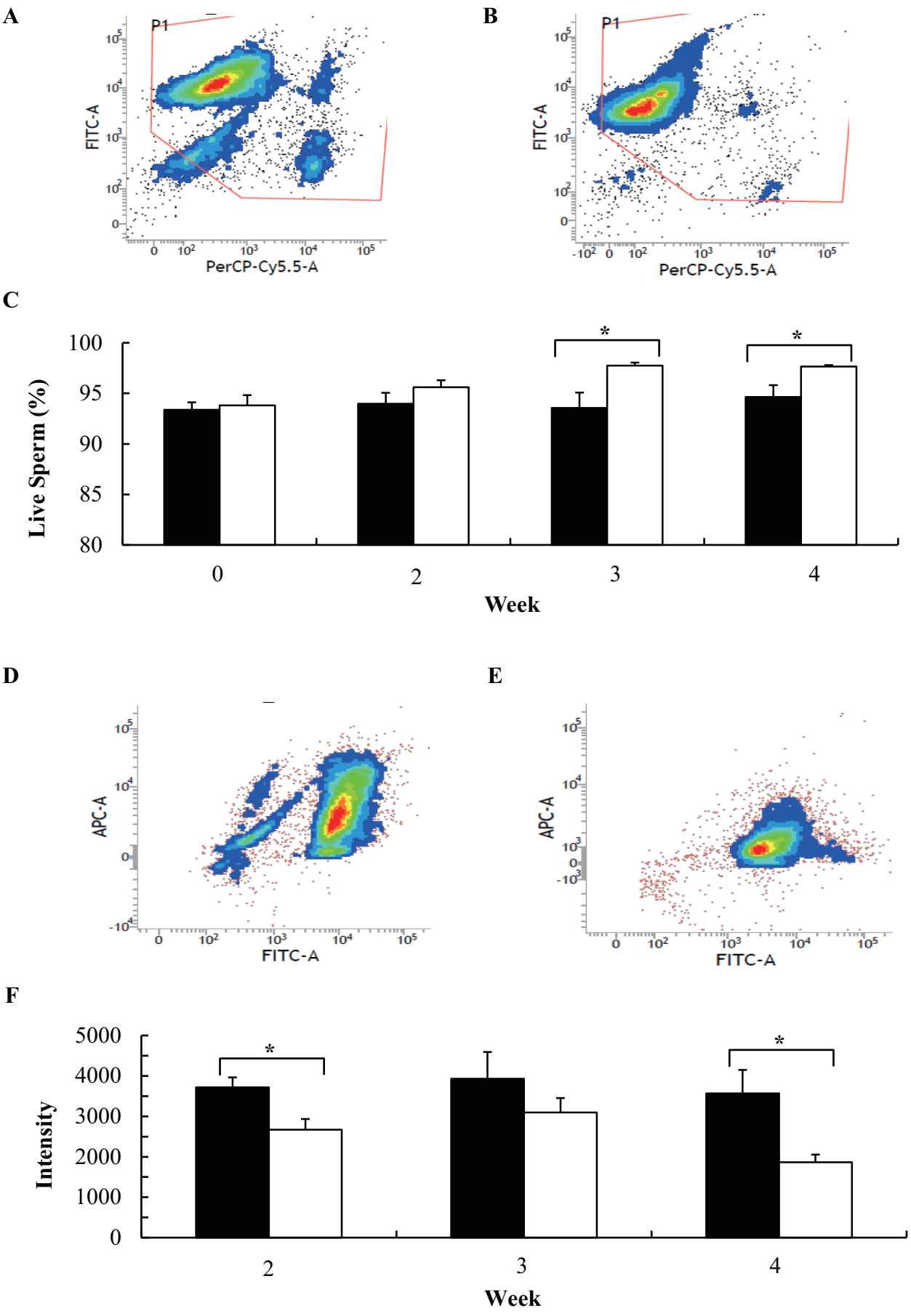

A
P1
FITC-A
PerCP-Cy5.5-A
B
P1
FITC-A
PerCP-Cy5.5-A
C
Live Sperm (%)
100
95
90
85
80
*
*
0
2
3
4
Week
D
APC-A
FITC-A
E
APC-A
FITC-A
F
Intensity
5000
4000
3000
2000
1000
0
*
*
2
3
4
Week

Figure 6

Conflict of interest: The authors have no conflicts of interest to disclose.