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Improvement of Reproduction Performance in AA$^+$ Meat-Type Male Chicken by Feeding with Squalene

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Abstract: In the present study, we investigated the effect of squalene on the reproductive performance in meat-type male chicken. A total of 480 meat-type males, which were 72 weeks old with body weight of 6.0±0.5 kg were assigned randomly and equally to 4 groups in two independental experiments which represent two classic mating types AI (Artificial Insemination) and NM (Natural Mating), respectively. Each experiment contains two groups, control group and squalene-treated group in which meat-type males were fed with the supplemental squalene at a daily dose 10 mg/kg/day for 45 days. Semen data such as average collection semen volume, sperm count and egg fertile rate were collected from experiment I that was designed keeping males and females seperated and using artificial insemination as mating type. In the experiment I that designed keeping birds together in floor and using natural mating as mating type, only fertility data were collected. Also, serum testosterone levels were measured in squalene-treated, control and males no ejaculation groups. In the experiment I, the average collection semen volume is 393 μL, which is significantly higher ($p<0.001$) than the control group, which is 287 μL. The egg fertile rate has no significant difference between these two groups. In experiment II, the egg fertile rate of squalene treated group was significantly higher than control group ($p<0.001$).The serum testosterone levels of squalene treated group was significantly higher ($p<0.05$) than control group. The results showed that meat-type males treated with squalene increased the serum testosterone level and semen collection volume and did not decrease egg fertile rate in AI model and also increased the egg fertile rate in natural mating model. These findings have implications that squalene has a prompt effect on reproductive performance on meat-type male chicken.

Key words: Chicken, meat-type male, squalene, testosterone, reproduction performance

INTRODUCTION

The breeding longevity of the meat-type male and female is different in the progress of the entire reproduction cycle. Because male reproduction performance traits, such as semen volume, semen quantity and sperm counts were lower for the elder meat-type males, fertility will decrease 10-20% in 2 weeks after mating 30 weeks. In another word, the breeding longevity of the meat-type male is shorter than female that will case huge loss for the owners of the breeding company without adjusting the structure of the males or finding a way to keep the fertility because those owners normally withdraw meat-type male breeders until the end of second laying period for economic reason in China. Although, replacing part of the old males in a flock with a set of new and younger males after 5-7 months of the egg production cycle can increase fertility, the procedure is costly and creates a disease risk (Bell and Weaver, 2002). Whether we can find a way to recover meat-type male's reproduction capability for increasing the breeding longevity instead of the traditional way to solve this problem is a useful project to animal science researchers. To reach this goal, some studies tried to keep meat-type male's reproduction capability with feed additions such as herbal medicine (Chinese herb feed additives, Qin and Liu, 2007), copper and zinc (Liu *et al*., 2006; Pitts *et al*., 1966). But it caused some problems such as short period acting, poisonousness as well.

Squalene (2, 6, 10, 15, 19, 23-hexamethyl tetracosah-eaxaene) is an isoprenoid compound with 6 isoprene units. It is the the cholesterol metabolism intermediateand

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that can be produced by biosynthesis or fermentation and exists widely in animals and plants (Zhao and Sun, 2004). Previous reports showed that squalene have diverse biological functions, such as preventing cell deterioration, antisenescence effect and enhancing immunity and sexual function (Zhang *et al*., 2008; Fang *et al*., 2004). Kamimura *et al*. (1989, 1991) showed that after feeding with squalene at 1200 mg kg^{-1} per day for a period of 14 days, there was no obvious adverse effect on dogs and it is widely used in medical care and cosmetics, thus squalene appears to be a safe compound. In recently study, feeding with supplemental squalene enhanced the reproduction performance in boars (Zhang *et al*., 2008). However, little has been published about the effect of squalene on reproduction performance of meat-type males. The objective of this study was to investigate the effects of feeding with squalene on the reproductive performance in meat-type males. Given that testosterone levels are associated with the reproductive performance of animals (Andre *et al*., 2008; Chun *et al*., 2003; Ortatatli *et al*., 2002; Somkuti *et al*., 1987; Kinger *et al*., 1995).

MATERIALS AND METHODS

Experimental animals: In the pre-experiment, meat-type males (n = 15) were randomly selected from a population of 480 individuals. Fifteen meat-type males were divided into 3 groups, treated with squalene at a daily dose 10, 20, 40 mg/kg/day for 2 weeks, respectively, average collection semen volume has no significant difference among these three groups. Thus we treated meat-type males with squalene at a daily dose 10 mg/kg/day as experiment groups.

Experiment I: Effect on reproduction performance in AA^+ meat-type males with feeding squalene by Artificial Insemination model: A total of 240 normal 72-weeks old AA meat-type males, with body weight of 6.0±0.5 kg were assigned randomly and equally to 2 groups, which allocated into squalene-AI (Artificial Insemination) group and control-AI group. Birds in squalene-AI group (n = 120) were fed with concentrated feed containing 10 mg/kg/day of squalene (Healthy Nature Resource, Inc, Walnut. CA 91789 U.S.A) for 45 consecutive days. Birds in 2 control groups (control-AI group, n = 120) were fed with concentrated feed without squalene. A total of 1600 AA hens were divided into 2 groups, e.g., 800 hens $group^{-1}$ to detect the fertility rate from semen collected from the above meat-type males. The nutrition composition of concentrated feed included 12.00% of protein, 0.64% of lysine, 0.90% of Calcium (Ca), 0.45% of Phosphorus (P), 0.49% of methionine and cysteine and 15 MJ kg^{-1} of energy. Meat-type males were housed and handled in accordance with the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching, First Edition, 1988.

Sperm count, semen volume and motility: During the experiment, only in AI groups, including squalene-AI group and control-AI group, semen samples were collected and recorded every 2 days for semen volume measurement, sperm counts and motility evaluation using normal methods. Average volume of every 20 meat-type males was calculated as one record unit.

Fertility: Eggs from AI hens of each meat-type male group were labeled and hatched. Fertility rate was recorded after 6 days.

Experiment II: Effect on reproduction performance in AA^+ meat-type males with feeding squalene by Natural Mating model: In this experiment, 240 normal AA meat-type males and 1600 hens come from the same flock as experiment I were randomly divided into 2 groups. This Natural Mating (squalene-NM, n = 120) group was fed with concentrated feed containing 10 mg/kg/day of squalene (Healthy Nature Resource, Inc, Walnut. CA 91789 USA) for 45 consecutive days and control group (control-NM group n = 120) was fed with concentrated feed without squalene, each group take care of total 800 hens by natural mating. Other conditions were the same as experiment I.

Fertility: Eggs from natural mating hens of each meat-type male group were labeled and hatched. Fertility rate was recorded after 6 days.

Experiment III: Blood and testosterone investigation: Meat-type males (n = 30, randomly selected in squalene treated group and control group) were blooded via the wing vein at the end of the experiment. Also meat-type males (n = 30) that could not ejaculate any semen were blooded. Serum samples were harvested and measured for testosterone levels using radioimmunoassay methods with ^{125}I-testosterone radioimmunoassay kits (coefficient of variation intra- and inter batch were <9.5%), which were provided by Beijing Kemei Dongya biologic technique Ltd. China).

Sexual and aggressive behavior: Meat-type males homo-copulation times, aggressive times and winner-retreat status were recorded in AI groups from 8:00-9:00 am every day during the experiment.

Data analysis: After 45 days experiment, data including the semen of control and experiment meat-type male sperm counts, semen volume and motility, fertility rate, serum testosterone concentrations and meat-type male sexual behavior were compared between the treated and control groups using students t-test.

RESULTS AND DISCUSSION

The sperm counts, semen volume and motility of squalene treated and control group of AI group meat-type males were shown in Table 1. Compared with control group, squalene treated group was significantly improved on sperm counts and semen volume ($p<0.001$) but no difference was detected on sperm motility. This result indicates that squalene can increase total sperm counts without harming sperm quality. The sperm counts was 1.8 fold higher in the treated group compared to the control group. Normally, semen volume from 100-1000 uL, e.g., 500 uL for average. Even through the average semen volume in squalene treated group did not reach 500 uL, it is close to it. Comparing with the traditional way, basically recover the elder males' reproduction capability by feeding with squalene is an economical and low risk model to solve the problem.

Table 2 has shown the fertility of the meat-type males from the experiment I with Artificial Insemination model. There are no differences on fertility between treated and control groups. The nomber of males that were used to collect sperm of the squalene-treated group is decrease 1/3 compared with control group. Table 3 has shown the fertility of the meat-type males from the experiment II with Natural Mating model. Under this condition, the fertility was significantly higher ($p<0.001$) for the squalene treated group compared to the control group, indicating squalene can improve fertility in natural mating groups.

Blood testosterone concentrations were investigated at the end of the experiment in the squalene-treated groups and control groups. In addition, 30 meat-type males that can not ejaculate were also investigated. The result were shown in the Table 4. The data suggested that there was significant difference ($p<0.05$) between squalene-treated groups and control groups. Squalene treated groups had significantly increased blood Testosterone level. This data also can explain why meat type males in squalene-treated groups can ejaculate more semen and have significant higher sperm counts than control groups. Egg fertility was significantly higher ($p<0.001$) in the squalene-treated group than control group in natural mating experiment. Blood testosterone concentrations of meat-type males that can ejaculate in both squalene-treated groups and control groups were significantly higher ($p<0.001$) than that of meat-type males that can not ejaculate any semen.

This result showed that blood testosterone concentration is an important parameter to measure meat-type male reproduction performance, although testosterone levels have variation among meat-type males in the same group overall experiments. Other studies also showed that testosterone levels are associated with the male reproductive performance in chicken and other animals (Andre *et al*., 2008; Chun *et al*., 2003; Ortatatli *et al*., 2002; Somkuti *et al*., 1987; Kinger *et al*., 1995; Zhang *et al*., 2008).

Meat-type males sexual and aggressive behavior data of AI groups were shown in Table 5. The aggressive times of squalene-treated group was significantly higher than the control group. The meat-type males in squalene treated group were more aggressive than the control ones, they always objectively attack other meat-type males. Meat-type males in squalene-treated group had significant higher frequencies to treat the small body size meat-type male as hens. During the experiment, there are three small body size meat-type males were died in the fighting in the squalene-treated group. This may be due to concentrations of testosterone in squalene treated group was higher and made the meat-type males more aggressive in sexual behavior and thus increased the fertility in nature mating groups. In the experiment I, there were approximately equal amount of semen inseminated to the hens, the sperm counts exceeded 8×10^8 (30 μL) only 1% normal sperms can enter the sperm tubulus, about 300-1000 sperm can penetrate to the ovum, 6-20 sperms can enter the oocytes and finish fertilization (Bakst *et al*., 1994). Whether the egg can be fertilized is determined by the capability and regulation of releasing sperms of the sperm storage tubulus of hen. Under nature mating, fertile egg rate was effected by the males' sucessful sexuall behavior, sexual agression and mating frequency. In the study, the meat-type males in the squalene-treated group

Table 1: Effects of squalene on sperm counts, semen volume and motility

Groups	Sperm counts (10^8 mL^{-1})	Semen volume (μL/male/time)	Sperm motility (%)
AI-s (10 mg kg^{-1})	45.86±2.26** (n = 18)	393.26±33.65** (n = 68)	83.78±7.61
AI-control	33.23±0.75 (n = 18)	287.61±19.33 (n = 68)	84.59±6.12

AI-squalene (10 mg kg^{-1}); **p<0.001 vs. control, Mean±SD; No * indicates no significant difference between two groups (p>0.05)

Table 2: Effects of squalene on fertility in AI groups

Groups	Total eggs	Fertilized eggs	Fertility (%)
AI-s (10 mg kg^{-1})	21748	17628	81.06
AI-control	23080	18729	81.15

AI-squalene (10 mg kg^{-1}), No* indicates no significant difference between two groups ($p>0.05$)

Table 3: Effects of squalene on fertility in NM groups

Groups	Total eggs	Fertilized eggs	Fertility (%)
NM-s (10 mg kg^{-1})	18776	12100	64.43**
NM-control	19180	11150	58.13

NM-squalene (10 mg kg^{-1}); **$p<0.001$ vs control

Table 4: Concentrations of testosterone in serum of meat-type males

Groups	Testosterone (ng dL^{-1})	Max. (ng dL^{-1})	Min. (ng dL^{-1})	C.V (%)
S-treated	346.35±261.18*	773.88	41.34	75.41
Control	240.20±178.61	578.46	4.34	74.36
No ejaculation	8.49±9.39	28.55	0.00	110.60

*$p<0.05$ vs. control, Mean±SD squalene-treated = s-treated

Table 5: Effects of squalene on meat-type male sexual behavior

Groups	Fight times	Homo-copulating	Fight result model
AI-s (10 mg kg^{-1})	5.33±1.04**	2.73±1.05**	Winner
AI-control	1.87±0.89	0.57±0.57	Retreat

AI-squalene (10 mg kg^{-1}); **$p<0.001$ vs. control, ±SD

had higher sexual passion sexual agression and fighting frequency in the AI group, thus it is suggest that feeding with squalene cause concentrations of testosterone inceeasing then cause sexuall behavior, sexual agression and mating frequency increasing in the squalene-treated nature mating group.

This result cause the fertility rate significantly higher than the control group. The results indicated that feeding 10 mg squalene kg^{-1} body weight can improve the reproduction performance of meat-type males. This result is very similar to the study of Zhang *et al.* (2008) in the pig.

On the other hand, this result is another evidence to support the idea that squalene enhances the productive performance in animals.

CONCLUSION

The study offered novel approach on improving the meat-type male reproduction performance and could be a practical way to reduce the cost of meat-type male breeding in chicken industry.

Taken together, squalene, as a safe, effective and economic (1000 g squalene cost USD 40 in China) feed additive can be used in intensive breeding farm to improve meat-type male reproductive performance in the future.

However, the detailed mechanism of the results is meriting further investigation.

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REFERENCES

Andre, O.G., R.A.P. Dornas, E. Kalapothakis, R.A. Hess, G.A.B. Cleida and M.A. Andre, 2008. Vitamin D3 and androgen receptors in testis and epididymal region of roosters (*Gallus domesticus*) as affected by epididymal lithiasis. Anim. Reprod. Sci., 109: 343-355.

Bakst, M.R., G.J. Wishart and J.P. Brillard, 1994. Oviductal sperm selection, transport and storage in poultry. Poult. Sci. Rev., 5: 117-143.

Bell, D.D. and W. Weaver, 2002. Commercial Chicken Meat and Egg Production. 5th Edn., Kluwer Academic Publishers, Amsterdam, The Netherlands, ISBN: 0-7923-7200-X, pp: 641-642.

Chun, L.F.W., M. Wei, X.Q. Li, Z. Liu, J. Yang and C.H. Hu, 2003. Fecal testosterone levels and reproduction cycle in male red panda (*Ailurus fulgens*). Acta Theriol. Sin., 23: 115-119.

Fang, X., W. Zhong and H. Miao, 2004. A study on the anti-senescense effect of squalene compound in rats anddrosophila melanogasters. J. Navy Med., 25: 289-291.

Kamimura, H., K. Fuchigami, H. Inoue, R. Kodama and H. Yoshimura, 1991. Studies on distribution and excretion of squalene in dogs administered for 2 weeks. Fukuoka Igaku Zasshi, 82: 300-304.

Kamimura, H., N. Koga, K. Oguri, H. Yoshimura, H. Inoue, K. Sato and M. Ohkubo, 1989. Studies on distribution, excretion and subacute toxicity of squalene in dogs. Fukuoka Igaku Zasshi, 80: 269-280.

Kinger, S., P.C. Pal, M. Rajalakshmi, P.K. Kumar, D.N. Sharma and J.S. Bajaj, 1995. Effects of testosterone buciclate on testicular and epididymal sperm functions in bonnet monkeys. Contraception, 52: 121-127.

Liu, J., X. Lang, Z.L. Hao and F.D. Li, 2006. Effect of dietary Zinc level on sermen quality and alkaline phosphatase in male chicken. Chinese J. Anim. Sci., 42: 20-22.

Ortatatli, M., M.K. Ciftci, M. Tuzcu and A. Kaya, 2002. The effects of aflatoxin on the reproductive system of meat-type males. Res. Vet. Sci., 72: 29-36.

Pitts, W.J., W.J. Millers and O.T. Fosgate, 1966. Effect of zinc deficiencies and restricted feeding from two to five months of age on reproduction in Holstein bulls. J. Dairy Sci., 49: 995-1000.

Qin, X. and F. Liu, 2007. Effects of Chinese herb additives on the sperm quality and ratio of fertilization in breeder cocks. China Feed, 16: 22-25.

Somkuti, G., D.M. Lapadula, R.E. Chapin, J.C. Lamb and M.B. Abou-Donia, 1987. Time course of the tri-o-cresyl phosphate-induced testicular lesion in F-344 rats: Enzymatic, hormonal and sperm parameter studies. Toxicol. Applied Pharmacol., 89: 64-72.

Zhang, W., Z. Xiong, D. Bi, X. Wang, Y. Cai, H. Dai and S. Chen, 2008. Feeding with supplemental squalene enhances the productive performance in boars. Anim. Reprod. Sci., 104: 445-449.

Zhao, Z. and Z. Sun, 2004. Research progress on natural resources and application of the bioactive substance squalene. Chem. Ind. For. Prod., 24: 107-112.