Effect of fructose addition in skim milk based extender on semen quality and fertility in white leghorn chicken

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Abstract

This experiment is designed to study the effect of fructose addition as energy source in skim milk extender on the quality fertility of white leghorn's spermatozoa stored at 4 °C. The treatments (T) in semen quality experiment were T1 (0% fructose + Modified Ringers Solution (MRS), T2 (5mM fructose + 1% (w/v) skim milk +MRS), T3 (10mM fructose + 1% (w/v) skim milk +MRS), T4 (15mM fructose + 1% (w/v) skim milk +MRS). In this experiment motility, morphology and in vitro sperm viability were examined at the 4^{th}, 8^{th}, and 12^{th} hour of storage representing four treatments each with three replications in 3X4 Factorial Completely Randomized Design. For the purpose of AI the semen is treated with T1 for immediate insemination within 30 minutes, with T2, T3 and T4 inseminated after 4 hours of storage and 360 eggs were incubated and examined for fertility, EM and hatchability. The result of this study indicates that semen characteristics of milky white color, mean volume of 0.36ml per ejaculation/rooster, mean pH of 7.2 and $5500X10^6$/ml mean concentration. There was significant difference ($P<0.05$) in sperm motility, morphology and in vitro viability in all fructose concentrations or control. There was significant difference ($P<0.05$) in fertility and hatchability between semen of different levels of fructose or control inseminated after 4 hours of storage at 4^oC. There was no significant difference in Early, mid and late EM (EEM, MEM and L EM) between semen of different levels of fructose or control inseminated after 4 hours of storage at 4^oC.

Keywords: Artificial Insemination, Fertility, Embryo, Hatchability, Semen, Skim milk

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Introduction

The development of artificial insemination (AI) technique has allowed the rapid dissemination of genetic material from a small number of superior sires to

a large number of females (Vishwanath and Shannon, 1997). Artificial insemination has been considered as a valuable technique in poultry industry. One of the advantages of this technology over natural mating is the efficient use of males. This in turn, decreases the cost of rearing males directly by reducing the number of cocks needed (Benoff *et al*., 1981).

The most common procedure for short-term fowl semen storage requires suspending sperm in an extender to retain their viability *in vitro* (Reddy, 1995). Addition of energy source in semen extender is required for proper functioning of the spermatozoa. Fructose appeared preferable to by inducing significantly higher percentages of motility compared to glucose and the mixture (Akhter, 2006). When fertility in the broiler breeds continues to decline due to the fact that males are selected for growth coupled with compatibility problems between large and smaller breeds, AI may become effective in broiler breeder management and in solving mating problems (Reddy, 1995). The possibility of dilution and storage of avian sperm would make the work of poultry breeders much easier, enabling them to transport semen even to distant farms, to inseminate large groups of females, and to improve the utilization of sperm from superior males.

The objective of this experiment was to determine the effect of extenders of different levels of fructose on the quality, fertility and hatchability of sperm originating from white Leg Horn chicken, and to evaluate if addition of fructose as energy source in skim milk extender can improve its ability to maintain the quality.

Materials and Methods

The experiment was conducted from December 2011 to May 2012 at Haramaya University Poultry Farm. The Semen was collected from 15 pure white leghorn males. Powdered skim milk, Fructose sugar (HIMEDIA RM 196-500g), antibiotics i.e. Gentamycin and Benzyl Penicillin (Kunming phar. Corp. P.R.C. BP 2000), where used in the semen extender. And Eosin and Nigrosin (Bio-Rad Laboratories, Inc.) staining was used to assess sperm morphology.

Extender Preparation

Table 1: Composition of extenders in the experiment.

S.N	Extender	Composition
1	T1 (Control)	Modified Ringers Solution (MRS) + 0% fructose + 0% skim milk (w/v)
2	T2	MRS + 5mM fructose + 1% Skim milk (w/v)
3	T3	MRS + 10mM fructose + 1% Skim milk (w/v)
4	T4	MRS + 15mM fructose + 1% Skim milk (w/v)

MRS: Modified Ringers solution, w/v: weight/volume

Antibiotics (Gentamycin @ 1 mg/ml and Benzyl Penicillin @ 1000 IU /ml), were added in each extender at room temperature.

Semen Collection and Evaluation

The semen was collected using Quinn and Burrows abdominal massage technique. After collection, the semen was transferred to laboratory for initial evaluation. Qualifying semen ejaculates were pooled to get sufficient semen for a replicate having motility >60% and were split into four aliquots for further processing. Semen aliquots were diluted at 37°C within 30 minutes with different extenders at 1:4 ratios for quality assay. For assessment of semen quality, treatments were formed from 4 levels of fructose and 3 levels of storage time in 4x3 factorial arrangements, and laid out in a completely randomized design (CRD) with 3 replications each.

More traditional semen evaluation procedures including determination of semen volume, color, concentration, motility, viability and morphology of spermatozoa were considered in this study. Progressive sperm motility was assessed microscopically (400X) and sperm morphology and viability were evaluated microscopically (1000X) using eosin-nigrosin under oil immersion.

Artificial Insemination (AI)

For this study, hens with similar age group (30 weeks of age) were used. The hens were kept in deep litter system fed with standard layers' ration.The vaginal AI developed by Quinn and Burrows was employed.

Treatments were composed of different levels of fructose and laid out completely at random with 3 replications each. Thirty female adult chickens were randomly selected and inseminated with extended semen without skim milk and fructose addition within 30 minutes after collection. And 90 female chick-

ens were also randomly selected and inseminated with semen stored for 4 hrs *in-vitro* at 4°C extended with 5mM, 10mM and 15mM of fructose levels,30 for each treatment (table 1). The insemination was done during afternoon because during the morning, most hens are believed have an egg in the oviduct,thus obstructing the free passage of semen to the ovary. 0.3 ml of semen, at intervals of 7 days was utilized. The Vaginal AI using a 1ml capacity sterilized syringe was performed.

Fertility, Embryonic Mortality and Hatchability

A total of 360 eggs were analyzed for Fertility, Embryo mortality and Hatchability using candling at 6th, 10th and 18th day of incubation and break-out analysis at 23rd day. Hatching eggs, were collected twice a day and un-cracked, clean eggs of at least 50g were marked and identified by pen number and treatment number, stored sharp point of egg downward, and pre-heated for 12 h at 25^{o}C prior to incubation. The eggs were set at random within racks and trays, in an 1100 eggs capacity incubator (pasrefrom BV hatchery tech. Zeddam, Holland) during 18 days at 37.5^{o}C (60-70% RH) and turned every hour at 90^{o}. Eggs were then placed in individual nets identified with pen and treatment number, prior to their transfer in a Hatcher (pasrefrom BV hatchery tech. Zeddam, Holland) during 3 days at 37^{o}C (60-70% RH).

At candling, clear eggs were removed, opened, inspected for evidence of embryo development. In the absence of an embryo, egg was classified infertile and for the fertile ones, embryonic mortality was described as Early (EEM) if observed at day 10, as Mid-Term (MEM) if observed at day 18 and as Late (LEM) if observed after day 21 if un-hatched. Break-out analysis was done at 23rd day of incubation analyzed for the development of eye or limbs or feather or the combination for the classification for Early EM, Mid-EM and Late EM.

Statistical Analysis

The data collected during the study period were subjected to one way Analysis of Variance using SAS computer software of version 9.1.3. Data collected on semen quality parameters were stratified into main factors (fructose and storage time) and interaction. Data collected on fertility, hatchability and embryonic mortality were stratified into fructose levels. When F-test was found significant, means were compared using LSD. A 5% ($P<0.05$) level of significance was used to determine statistical significance.

Results

General Semen Characteristics

Individual differences in terms of semen characteristics and sperm quality were not addressed in this study. The average volume, color and texture, concentration and pH of semen collected from trained white leghorn cockerels are shown below (Table 2).

Table 2: Semen characteristics of white leg horn cockerel.

Mean semen Characteristics	White leg horn Cockerel
Semen Volume (ml/ejaculate)	0.36ml
Color and Texture	Milky white and viscous
Sperm Concentration ($X10^6$/ml)	$5.5X10^9$
Sperm number/ejaculation	$1.98X10^9$
pH	7.2

Effect of Levels of Fructose concentration and Storage Time on Semen Quality

The effect of fructose addition, duration of storage and their interaction on motility, *in vitro* longevity and morphology of WL spermatozoa are presented in Table 3 and 4.

Table 3: Least square mean (±SE) percentage semen quality parameters as influenced by addition of fructose and storage time in white leg horn cockerels' semen

Factor	Motility (%)	Morphology (%)	*In vitro* viability (%)
Fructose	**	**	**
C	75.6±2.94^b	87.1±0.72^a	67.3±3.61^b
5mM	78.9±2.00^b	82.3±0.76^b	64.4±2.74^b
10mM	85.6±1.76^a	85.7±0.50^a	75.0±1.21^a
15mM	75.6±3.38^b	79.9±1.95^b	69.2±2.50^a
Storage time	**	**	**
4H	85.0±1.51^a	86.5±0.50^a	76.0±0.62^a
8H	80.8±1.47^b	83.4±1.15^a	71.4±1.37^b
12H	70.8±2.28^c	81.3±1.47^b	59.6±2.04^c

Table 4: Least square mean (±SE) percentage semen quality parameters as influenced by interaction of addition of fructose and storage time in white leg horn cockerels' semen.

Factor	Motility (%)	Morphology (%)	*In vitro* viability (%)
Fructose* storage time	NS	**	**
C*4H	83.3±3.33	88.3±0.33^{a}	76.7±1.45^{a}
5mM*4H	83.3±3.33	84.3±0.33^{b}	73.7±0.88^{a}
10mM*4H	90.0±0.00	86.3±0.88^{a}	77.7±0.88^{a}
15mM*4H	83.3±3.33	87.0±0.58^{a}	76.1±0.58^{a}
C*8H	76.7±3.33	87.0±1.53^{a}	72.0±0.58^{a}
5mM*8H	80.0±0.00	83.0±0.58^{b}	64.7±0.88^{c}
10mM*8H	86.7±3.33	85.7±1.20^{a}	77.0±0.58^{a}
15mM*8H	80.0±0.00	78.0±1.15^{c}	72.0±1.15^{a}
C*12H	66.7±3.33	86.0±1.53^{a}	53.3±1.20^{d}
5mM*12H	73.3±3.33	79.7±0.88^{b}	55.0±1.15^{d}
10mM*12H	80.0±0.00	85.0±0.58^{a}	70.3±0.33^{b}
15mM*12H	63.3±5.77	74.7±1.76^{c}	59.7±0.88^{c}

The effect of fructose concentration and storage time on sperm motility Morphology and *in vitro* sperm viability of white leg-horn chicken is presented in Table 3. There was significant difference (P>0.05) in sperm motility among all fructose levels considered in this study. There was significant difference (P<0.05) in sperm abnormalities across the durations of storage considered and in extenders with different levels of fructose supplementations. There was, significant difference (P<0.05) in sperm *in vitro* viability at 8th and 12th hour storage in extenders with different levels of fructose supplementations or control.

Fertility and Hatchability

The effect of fructose concentration on fertility and hatchability of white leg-horn chicken is presented in Figure 1.

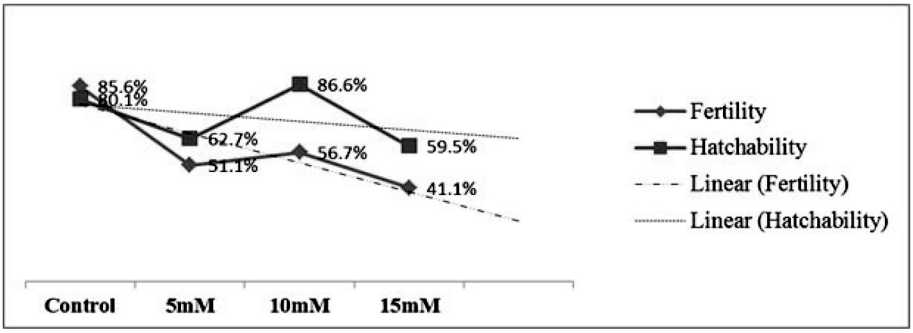

Figure 1. Effect of fructose addition on fertility and hatchability of white leg horn's semen stored *in vitro* and inseminated after 4 hours of storage

Semen containing the control (MRS) extender was significantly different ($P<0.05$) in sperm fertility from semen of different levels of fructose and there was no significant difference ($P<0.05$) in sperm fertility between extenders containing 5mM, 10mM and 15mM fructose concentrations. Extender containing the control treatment (MRS)shows the highest fertility level (85.6±11.28). Extender containing 15mM fructose concentrations shows the least fertility level (41.1±4.01).

Embryonic mortality (EM)

There was no significant difference ($P>0.05$) between semen treated with different levels of fructose in all stages of embryonic mortality (EM). The effect of fructose concentration on embryonic mortality of white leg-horn chicken is presented in Figure 2.

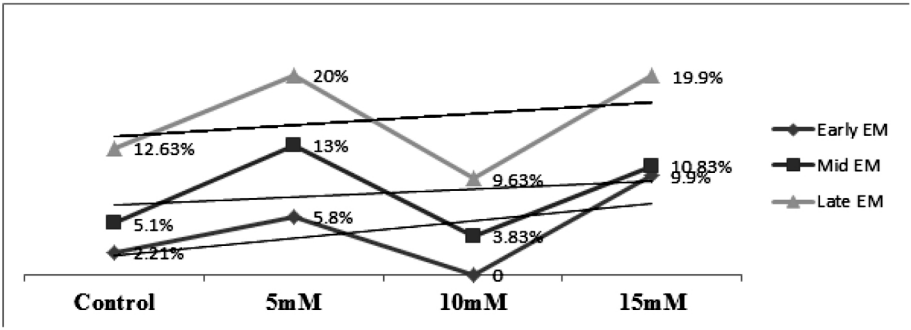

Figure 2. Effect of fructose addition on embryonic mortality of white leg horn's semen stored *in vitro* and inseminated after 4 hours of storage.

Discussion

Semen volume recorded in this study was within the range of 0.1 ml to 1.5 ml per ejaculation in agreement with that of Cole and Cupps (1977). The average volume of ejaculated semen using abdominal massage technique was 0.25ml (Gordon, 2005) and 0.28 ± 0.14ml (Bah *et al.* 2001). On the other hand Hafez and Hafez (2000) indicated that the average sperm volume collected from white leghorn varies 0.2-0.5 which is also in agreement with the result found in this study.

The color of semen may depend on the species of bird used; but generally semen should be creamy which indicates a high sperm concentration (Cole and Cupps, 1977). The average sperm concentration in the present study was $5500X10^6$/ml (Table 2). The sperm concentration from the present study ($5500X10^6$/ml) is within the range of a report by Hafez and Hafez (2000), which is $3000\text{-}7000X10^6$ spermatozoa/ml. The average pH of the semen collected was slightly alkaline and ranges from 7.10 to 7.30 (Table 2). These results are all within the range generally reported for white leg-horn semen by Hafez and Hafez (2000). The accessory sex gland fluid is generally alkaline (Bah *et al.*, 2001; Peters *et al.*, 2008).

Sperm Motility, Morphology and *In vitro* viability

As reported by Ponglowhapan *et al.* (2004) motility is an important indicator of sugar utilization by spermatozoa as sugars serve as an external energy source essential for maintaining motility. This study demonstrated that semen extended with skim milk based extender containing 10mM fructose and stored at 4 hours produced higher sperm motility (Table 3 and 4). In this study the overall average sperm motility was 78.8%, which is in general agreement with 60-80% reported by Hafez and Hafez (2000).

In this study, extending semen with skim milk based extender without fructose but with MRS (control) and storage of extended sperm at 4 hours yielded least sperm abnormalities. Tselutin *et al.* (1999) reported the number of live sperm without any abnormalities in cockerel semen to vary from 91 to 94%, which is higher than the results of this study. But Tuncer *et al.* (2006) reported that the number of abnormal sperm in cockerel semen to vary from 9.2 to 11.23%, which is in agreement with sperm abnormalities recorded in control treatment.

Extender containing 10mM fructose concentration and 4 hours of storage is the best combination (77.67±0.88%) for better *in vitro* sperm viability (Figure 4). The addition of fructose will not greatly change the metabolic rate, but will extend the life span of the sperm (Bearden *etal.,* 2004). According to the report by Gebriel *et al.* (2009), 81.79% sperm *in vitro* viability was recorded, which is in agreement with the result of present study.In this study, the percentage of dead sperm increased by 14.17% over 12 hrs of storage and was positively correlated with the storage time.

Fertility, hatchability and embryonic mortality

Fertility and hatchability are major parameters of reproductive performance which are most sensitive to environmental and genetic influences (Stromberg, 1975). Heritability estimates for fertility and hatchability in chickens range from 0.06-0.13 (Sapp *et al*., 2004), this indicates that the non-genetic factors have a higher influence on these traits.

A study by Alsobayel (1992) indicated higher fertility (95%) can be maintained under good management which is far greater than fertility level of the present study (58.6±6.13). In addition to semen quality several factors affect fertil-

ity such as; nutritional factors, bird factors, egg factors and factors associated with insemination procedure (King'ori, 2011).

The reasons for embryo mortality during incubation are complex. There are many factors contributing to the failure of a fertile egg to hatch which include lethal genes, insufficient nutrients in the egg and exposure to conditions that do not meet the needs of the developing embryo (Kirk *et al*., 1980). The present study is partially in agreement to the report of Máchal *et al*. (2003) which states the embryonic mortality of all the lines was the highest during the early stage of incubation, i.e. 45.1% (within 4 days 36.9% and within 7 days another 8.2%). The embryonic mortality of all the lines was the lowest in the mid stage of incubation – within 14 days (13.4%). In the third trimester and in the period of breaking through the shell the embryonic mortality was again high, i.e. 41.5% (within 20 days it was 23.4% and in the period of breaking through the shell another 18.1%) in different strains of chicken.

Conclusion

Addition of energy source in semen extender is required for proper functioning of the spermatozoa. In this study, fructose as energy source can improve motility and *in vitro* longevity. But sperm morphology was declining as the concentration of fructose increases. Immediate insemination of semen extended with the control treatment is recommended for better fertility and hatchability. Further investigation is needed for the causes of Embryo mortality in all extenders used in this experiment.

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