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Powdered coconut water as preservant of semi-heavy cocks semen

Água de coco como preservante de sêmen de galos semipesados

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SUMMARY

This study aimed to evaluate the effect of powered coconut water as preservant of cocks semen in different times. The experimental method was completely randomized, with treatments constituted by different times (1, 5, 10 and 15 minutes) of powered coconut water as preservant of cocks semen (Rhode Island Red lineage with 30-weeks). After collect and use of powered coconut water as preservant, the semen samples were used for inseminated of breeders of same lineage and age (16 per treatment). 280 eggs were collected for evaluation of effects on chicks (70 eggs per treatment). Were evaluated the incubation yields, chick/egg relation and gastrointestinal tract development of chicks. The data collected were submitted for variance analysis and evaluated by Tukey test at 5% of significance. Differences ($p<0.05$) were observed in incubation yields, chick/egg relation and gastrointestinal tract development, with the exception ($p>0.05$) of oropharynx + oesophagus length and yolk sac weight. From these results, it was concluded that the powered coconut water can be used as preservation of cocks semen up to 15 minutes. Cells exposed more time to nutrients provided by the powdered coconut water showed eggs and chicks most heavier, and better results in the incubation yields and embryo development.

Keywords: biotechnology, embryo development, fertility, preservation

RESUMO

O objetivo com este estudo foi avaliar o efeito da água de coco como preservante do sêmen de galos em diferentes períodos. O delineamento experimental foi inteiramente casualizado, onde os tratamentos consistiam de diferentes períodos (1, 5, 10 e 15 minutos) de ação da água de coco como preservante do sêmen de galos (linhagem Rhode Island Red com 30 semanas). Após a coleta e submissão do sêmen a ação da água de coco, estas foram utilizadas para inseminação de 64 matrizes de mesma linhagem e idade (16 por tratamento). Foram coletados 280 ovos (70 ovos por tratamento) para avaliação dos efeitos da preservação do sêmen sobre a progênie. Foram avaliados os rendimentos de incubação, relação pinto/ovo e o desenvolvimento do trato gastrointestinal dos pintainhos. Os dados coletados foram submetidos à análise de variância e avaliados pelo teste de Tukey a 5% de significância. Diferenças ($p<0,05$) foram observadas nos resultados dos rendimentos de incubação, relação pinto/ovo e desenvolvimento do trato gastrointestinal, à exceção ($p>0,05$) dos resultados de comprimento da orofaringe + esôfago e peso do saco vitelino. A partir dos resultados obtidos, concluiu-se que a água de coco em pó pode ser utilizada como preservação do sêmen de galos até 15 minutos. As células expostas por mais tempo aos nutrientes da água de coco em pó proporcionaram ovos e pintos mais pesados, e melhores resultados nos rendimentos de incubação e desenvolvimento embrionário.

Palavras-chave: biotecnologia, desenvolvimento embrionário, fertilidade, preservação

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INTRODUCTION

The biotechnology has several areas of study, especially the use of techniques for collect, evaluation and preservation of semen to artificial insemination and evaluation of animal progeny. And its application in the poultry industry, mainly due to the easy management, can to help the use of artificial insemination, besides providing the development of efficient procedures to preserve the semen in vitro conditions for long periods (RUTZ et al., 2007; BONGALHARDO et al., 2009; RUFINO et al., 2014; FEIJÓ et al., 2016).

Semen preservation represents an excellent support for poultry industry, maying be used in breeding selection programs. But, this tool is still few used on an industrial scale, mainly in the reproductive management of cocks (MASSIP et al., 2004; MORAIS et al., 2012), even the intense investment in preservation research with poultry semen (BONGALHARDO, 2013; PARTYKA et al., 2012).

Is large the list of diluents, preservatives, protectants and different protocols used to improve the poultry semen performance, standing out the use of powered coconut water. This product was tested, and proved that can to maintain the fundamental properties of the natural seminal fluid, showing stability and longevity (important features for industry), replacing chemical and expensive products (MOREIRA-NETO et al., 2009; PURDY et al., 2009; SOARES & GUERRA, 2009; BONGALHARDO, 2013).

Coconut water presents as a sterile and slightly acidic natural solution composed by proteins, salts, sugars, vitamins, neutral fats, besides cell division inducers and various electrolytes, which confer density and pH compatible with blood plasma, providing nutrients needed to maintain the survival and viability of male and female gametes preserved (RONDON et al., 2008; LAVOR & CÂMARA, 2012).

According the above, this study aimed to evaluate the effect of powered coconut water as preservant of cocks semen in different times.

MATERIAL AND METHODS

This study was conducted at the Laboratory of Poultry Technology, Poultry Sector, Department of Animal and Vegetable Production (DPAV), College of Agrarian Sciences (FCA), Federal University of Amazonas (UFAM), South Sector at the University Campus, Manaus, State of Amazonas, Brazil.

32 breeder cocks Rhode Island Red (32-weeks and average body weight 2.05±0.12) identified and with proven fertility were used. These were housed in boxes with 4 m² in density of 1 bird/m^2, with food and water *ad libitum*.

The experimental method was completely randomized, with treatments constituted by different times (1, 5, 10 and 15 minutes) of powered coconut water as preservant of cocks semen.

Individual collects of semen were performed according to the methodology proposed by Burrows & Quinn (1937), using the method of abdominal massage in back and movements on the sides of cloaca.

A *pool* of ejaculates was made from the formation of solution with semen and diluent (Beltsville Poultry Semen Extender (BPSE) - BOOTWALLA & MILES, 1992), being shared into four large samples that were subjected to the action of powdered coconut water (ratio

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of 10 to 1) in predetermined periods (one sample per period).

16 female breeders per treatment were inseminated by the method of abdominal massage in back and deposition of semen directly into the oviduct. These female breeders were same lineage and age of breeder cocks, and were housed in cages.

280 eggs were collected (70 eggs per treatment) for evaluation of effects of preservation of semen on the progeny. These were fumigated, identified, weighed and distributed in an incubator machine PETERSIME 168 with 37.6 ºC temperature, 66% relative humidity and turn of eggs at one-hour intervals.

At 19 days of incubation, the eggs were weighed and evaluated for separation of infertile eggs or dead embryos. The fertile eggs were transferred to hatching machine PETERSIME 168 with 36.6 ºC temperature, 76% relative humidity at 21 days of incubation (504±2 hours).

After birth, were registered and weighted the chicks hatched for evaluation of incubation yields and chick/egg ratio. Then, five chicks per treatment (where there was sufficient birth) were slaughtered for evaluation of heart (g) and gastrointestinal tract development (liver (g), yolk sac (g), pancreas (g), gizzard (g), pro-ventricle (g), digestive system length (cm), oropharynx + esophagus (cm), duodenal loop (cm), jejunum + ileum (cm), cecum (cm) and colon + rectum (cm)).

Statistical analysis was performed using the software Statistical Analysis System (2008), and estimates of treatments were subjected by Tukey test at 5% of significance.

RESULTS AND DISCUSSION

Differences ($p<0.05$) were observed in chick/egg relationship, where eggs and chicks from breeders inseminated with semen subjected to preservation using powdered coconut water after 15 minutes showed better results (Table 1).

Table 1. The effects of powdered coconut water for semen preservation on chick/egg relationship

Semen preservation times	Variables			
	Egg weight (g)	Egg weight loss (%)	Chick weight (g)	Chick/egg correlation
1 minute	49.84^{b}	8.48^{bc}	30.54^{b}	0.67^{b}
5 minutes	50.36^{ab}	9.65^{b}	0.00^{c}	0.00^{c}
10 minutes	50.40^{ab}	10.82^{ab}	32.84^{ab}	0.73^{ab}
15 minutes	50.54^{a}	12.03^{a}	33.50^{a}	0.75^{a}
p-value	0.05	0.01	0.01	0.01
Effect	*	*	*	*
CV (%)	1.52	11.37	11.27	11.70

CV = coefficient of variation; p-value = coefficient of probability.
*Means followed by lowercase letters in column differ in 5% by Tukey test ($P<0.05$).

Naturally, the semen preservation procedures aim to reduce the damage caused during long storage periods or over long distances transport (RONDON et al., 2008). However, there is a large lack of information about its effects on semen used in the chicks production,

especially the possible interferences in the quality of this animals.

In this study, it was observed that in the semen exposed to the preservant (powdered coconut water), there was an increase in all observed indexes of chick/egg relationship. And the use of powdered coconut water may allow a longer period to use the collected semen aliquots, with a better use and a possible of higher number of females to be inseminated.

Differences ($p<0.05$) were observed also in fertility, hatchability and hatching (Table 2), with positive sperm answer from increase of semen preservation period.

A sensitivity in the sperms may have occurred, causing an immediate mortality of the cells due any factors, how a very short period of exposure to the nutrients, difficulty of cells adaptation to osmolarity, natural development of sperms and others.

Table 2. The effects of powdered coconut water for semen preservation on chick/egg relationship

Semen preservation times	Variables		
	Fertility (%)	Hatchability (%)	Hatching (%)
1 minute	67.14[b]	46.72[b]	31.43[b]
5 minutes	0.00[c]	0.00[c]	0.00[c]
10 minutes	41.43[ab]	79.28[ab]	32.86[ab]
15 minutes	75.71[a]	86.64[a]	65.71[a]
p-value	0.02	0.01	0.01
Effect	*	*	*
CV (%)	9.56	4.02	14.25

CV = coefficient of variation; p-value = coefficient of probability.
*Means followed by lowercase letters in column differ in 5% by Tukey test ($P<0.05$); ns = non significant.

However, when the sperms were exposed for a longer period (after 5 minutes) to the nutrients, there was sufficient time to stabilize in the exposed medium (diluent + powdered coconut water) and its environment conditions.

According Fontana et al. (1990), the good semen have to contain at least 80% of normal sperms, and a maximum of 20% of sperms with abnormalities. But, when this margin is extrapolated, the results are directly reflected in the percentages of egg fertility and birth of chicks (RUTZ et al., 2007; RUFINO et al., 2015; BEZERRA et al., 2015; RUFINO et al., 2018).

Cardoso et al. (2007) also affirm that from the use of semen preservation procedures, it can obtained a larger number of viable sperms with better quality for expressed its all potential during the process of egg fertilization.

In results of heart and gastrointestinal tract development, differences ($p<0.05$) were observed for organs (Table 3), and regions (Table 4), with better embryo development from breeders inseminated with preserved semen for 15 minutes.

In agreement with the results of Table 1, chicks from eggs of breeders inseminated with semen that remained longer exposed to the preservant presented greater embryo development.

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Table 3. The effects of powdered coconut water for semen preservation on gastrointestinal development (organs)

Semen cryopreservation times	Variables					
	Yolk sac (g)	Heart (g)	Liver (g)	Pancreas (g)	Pro-ventricule (g)	Gizzard (g)
1 minute	2.38	0.62^{ab}	1.37^{ab}	0.12^{bc}	0.61^{ab}	2.23^{bc}
10 minutes	2.34	0.61^{ab}	1.48^{ab}	0.31^{ab}	0.63^{ab}	2.31^{ab}
15 minutes	2.29	0.82^{a}	1.74^{a}	0.42^{a}	0.78^{a}	2.55^{a}
p-value	0.90	0.01	0.01	0.02	0.04	0.03
Effect	ns	*	*	*	*	*
CV (%)	17.23	17.28	14.63	14.77	10.49	19.16

CV = coefficient of variation; p-value = coefficient of probability.
*Means followed by lowercase letters in column differ in 5% by Tukey test (P<0.05); ns = non significant.

Table 4. The effects of powdered coconut water for semen preservation on gastrointestinal development (regions)

Semen preservation times	Variables					
	Gastrointestinal tract (cm)	Oropharynx + oesophagus (cm)	Duodenal loop (cm)	Jejunum + ileum (cm)	Cecum (cm)	Colon + rectum (cm)
1 minute	43.06^{b}	6.60	4.72^{b}	23.98^{b}	3.60^{b}	7.70^{b}
10 minutes	48.20^{ab}	6.16	5.80^{ab}	24.00^{ab}	4.50^{ab}	9.00^{a}
15 minutes	53.40^{a}	6.70	6.40^{a}	30.20^{a}	4.70^{a}	9.00^{a}
p-value	0.01	0.37	0.05	0.03	0.03	0.02
Effect	*	ns	*	*	*	*
CV (%)	9.27	9.62	8.29	14.65	14.32	16.78

CV = coefficient of variation; p-value = coefficient of probability.
*Means followed by lowercase letters in column differ in 5% by Tukey test (P<0.05); ns = non significant.

These also may be related to the better quality that the semen can acquire from contact with the nutrients of powdered coconut water. Rondon et al. (2008) affirm that the use of a nutrient-rich diluent capable of maintaining the isotonicity of the medium (isotonicity and osmolarity) and pH without compromising the membrane of sperms is important for the reproductive processes, aiming a better development of the embryo.

From these results, it was concluded that the powered coconut water can be used as preservation of cocks semen up to 15 minutes. Cells exposed more time to nutrients provided by the powdered coconut water showed eggs and chicks most heavier, and better results in the incubation yields and embryo development.

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