Feed supplementation with palm kernel cake-based concentrate increases the quality of water buffalo semen

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Abstract

Feed supplementation can represent a relevant strategy to raise reproductive indexes in water buffalo herds. When diets rich in lipids are offered to ruminants, there is a benefit to reproduction. Considering that palm kernel cake (PKC) and coconut meal (CM) are lipid-rich industrial by-products, this study aimed to evaluate the effect of concentrates made of PKC or CM on the nutrient intake by water buffalos (*Bubalus bubalis*), to investigate their effects on semen characteristics, and to correlate semen parameters to the supplementary intake of lipids, crude protein, and minerals. Fifteen water buffalos kept on pasture were used as semen donors. The animals received daily dietary supplementation (1% of body weight) for 252 days, and were divided into three groups: Control (n = 5; conventional concentrate), CM-Base (n = 5; coconut meal concentrate), and PKC-Base (n = 5; palm kernel cake concentrate). Aspect, color, volume, pH, gross motility, vigor, progressive sperm motility, plasma membrane integrity, and sperm morphology of ejaculates were evaluated (173 samples). The daily average for voluntary concentrate intake was 4.778 ± 1.233 kg in the Control group, 3.112 ± 0.693 kg in CM-Base, and 4.558 ± 1.077 kg in PKC-Base group (P > 0.05). Diet based on palm kernel cake supplementation had a positive effect on semen quality. The plasma membrane integrity levels were increased in PKC-Base animals (Control: 68.0 ± 19.5; CM-Base: 72.0 ± 22.6; PKC-Base: 82.1 ± 12.2; P < 0.05). In addition, progressive sperm motility was higher in the PKC-Base group ($71.7 \pm 15.1\%$ PKC-Base; $59.3 \pm 20.5\%$ Control; $56.7 \pm 24.8\%$ CM-Base; P < 0.05). Lower sperm concentration values were observed in the PKC-Base group, but they did not deviate from the physiological standards from 524.10 ± 20.70 to $1,031.4 \pm 28.70$ x 10^6 sptz/ml, or of $1,493$ x 10^6 sptz/ml. The other sperm parameters did not have significant differences (P > 0.05). Hence, palm kernel cake-based dietary supplementation for water buffalos provided more lipids and minerals, which intake is related to the improvement of sperm quality, with higher sperm motility and higher levels of spermatozoa with plasma membrane integrity.

Introduction

Feed exerts great influence on animal reproduction (Boland et al., 2001; Barth *et al*., 2008) and nutrients have specific influence mechanisms on the reproductive system and the functioning of the endocrine system (Robinson *et al*., 2006). Due to this interrelation, animal feed supplementation represents an important strategy to raise reproductive and productive indexes in water buffalo herds (Dimri *et al*., 2010; Ranjan *et al*., 2012). From the productive and environmental points of view, animal feed based on the reuse of agro-industrial residues could be a viable bioeconomic alternative for sustainable animal husbandry.

In the tropics, water buffalos (*Bubalus bubalis*) are raised where coconut (*Coconuts nucifera*) and palm oil trees (*Elaeis guineensis* Jacq.) are relevant commodities and the growing production is used in the food and biofuel industries (Joele *et al*., 2012). Coconut meal is defined as a by-product from the extraction of coconut oil, by means of maceration and pressing, made from the coconut pulp or from the dried copra. The oil content from coconut meal can vary according to the extraction method, and the crude protein content in the meal ranges from 20 to 25%. Coconut meal also contains 8.8% ether extracts, 46.7% neutral detergent fiber, and 5,461.06 kcal/kg gross energy (Souza Júnior *et al*., 2009). On the other hand, palm kernel cake is the by-product from the milling and extraction of the oil from the dry pulp kernel. It can be used for animal feed and has from 11.9 to 14.5% crude protein, 7.2 to 12.0% ether extracts, 81.8% neutral detergent fiber, and 1.5% non-fibrous carbohydrates (Silva *et al*., 2005). Both coconut meal and the palm kernel cake can potentially replace conventional feed ingredients, such as corn and soybean, for ruminants, especially in critical periods of

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the year, when production costs rise.

When lipid-rich diets are offered to ruminants, there is a benefit to reproduction, even though different responses may be caused by the difference in fatty acid profiles in the supplements and their availability to the tissues (Thatcher *et al.*, 2011). Despite the intense biohydrogenation suffered by polyunsaturated fatty acids in the rumen, their levels increase in many tissues after dietary supplementation, including in the gonads (Bilby *et al.*, 2006). Lipids are constitutive molecules of the sperm plasma membrane. They play a very important role in determining membrane fluidity, as well as in sperm motility and cell viability. In the seminal plasma, lipids are also important for spermatozoa protection because they participate in the antioxidative processes that occur in ejaculates (Kelso *et al.*, 1997), and in the process of sperm capacitation (Kadirvel *et al.*, 2009). Since they have high lipid content, palm kernel cake and coconut meal may, hypothetically, be added to the feeds given to water buffalo bulls, but the consequences of their use on semen quality are not known. Thus, this paper aimed to evaluate the effect of concentrates made of palm kernel cake or coconut meal on the nutrient intake by experimentally fed water buffalos to study their effects on semen characteristics and to correlate semen parameters to the supplementary intake of lipids, crude protein, and minerals.

Material and Methods

Location and experimental animals

Fifteen adult bulls, belonging to the Embrapa Eastern Amazon herd, in Belém, Pará, Brazil (1°28′S, 48°27′W) were used in the study. The animals had been previously selected according to their andrological evaluations (Colégio Brasileiro de Reprodução Animal - CBRA, 1998), which were positive in the beginning of the trial. Their mean age was 3.2 ± 1.3 years, average weight 578.6 ± 101.9 kg, and they had similar andrological status. The bulls were equally divided into three experimental groups (Control, n = 5; CM-Base, n = 5; PKC-Base, n = 5) and kept in a single lot for pasture. The experimental area used consisted of 5.4 ha, cultivated with *Panicum maximum* cv. Mombaça (guinea grass), divided into five paddocks for intensive rotational grazing (3.23 AU/hectare), which was arranged into 30-day grazing cycles, being six days of occupation per paddock and 24 days of rest. The animals had free access to drinking water. All the procedures adopted observed the bioethical principles recommended for animal experimentation (Paixão, 2005) and the research protocol had been previously approved by the Animal Research Ethics Committee at the Federal University of Pará (CEPAE/UFPA - Approval BIO22-10). The experiment lasted for 252 days, divided into nine periods (P1 to P9) of 28 days each.

Animal feed supplementation and intake

Three experimental concentrates were made from agro-industrial by-products in order to be isoproteic (~18% crude protein), according to the formulation below: Control (concentrate made up of 62.4% ground corn, 24.8% soybean meal, 11.9% wheat meal, 1.0% limestone); CM-Base (concentrate made up of 18.8% ground corn, 69.3% coconut meal, 10.9% wheat meal, 1.0% limestone); and PKC-Base (concentrate made up of 2.0% ground corn, 69.3% palm kernel cake, 14.9% soybean meal, 12.9% wheat meal, 1.0% limestone). The bromatologic composition of the experimental concentrates is found in Table 1.

Table 1. Bromatological composition of experimental concentrates formulated based on industrial by-products.

Parameter	Group[*]		
	Control	CM-Base	PKC-Base
Bromatological composition (%)			
Dry matter	87.13	91.63	90.97
Crude protein	18.46	18.21	18.89
Neutral detergent fiber	19.83	45.42	63.87
Acid detergent fiber	11.65	28.84	35.84
Ether extracts	3.64	8.87	11.82

[*]Control (based on ground corn and soybean meal), CM-Base (based on coconut meal), and PKC-Base (based on palm kernel cake).

Concentrate was supplied daily in covered pens with individual troughs and yokes, so that each animal received an amount of concentrate equivalent to 1% of their body weight (BW) every morning. The amount supplied was adjusted every 28 days, according to the animals' weight evolution. The animals were weighed individually using a large animal electronic scale after a 12-hour fast. Mineral mix was offered in the troughs, at the amount of 100 g/head/day. The daily concentrate intake was calculated individually, by measuring the difference between the offered amount and leftovers, which were surveyed after a period of time long enough for all the animals to be fed and satisfied. After the concentrate intake, the animals were led back to the pasture area, where they remained until their feeding on the following day. Dietary supplementation was carried out throughout the experimental period, and P1 was used for the animals' adaptation to the formulated diets.

Supplementary intake of crude protein, lipids, macronutrients (Ca, Na, Mg, and K) in g/day and micronutrients (Fe, Mn, Mo, Co, Cr, Cu, Zn, and Se) in mg/day was determined based on the identified contents of each element and on the daily individual concentrate intake.

Supplement evaluation

The supplements offered were analyzed regarding dry matter (DM), crude protein (CP), ether extracts (EE), neutral detergent fiber (NDF), and acid detergent fiber (ADF), according to the methodology described by Silva and Queiroz (2002). For the analyses of fatty acids profiles, samples were lyophilized and subjected to fat extraction. Acid esterification was performed according to the methodology recommended by the Association of Official Analytical Chemists - AOAC (2005). Retention data of esters were analyzed by gas chromatography (CP 3380, Varian Analytical Instruments, Walnut Creek, U.S.A.), with flame ionization detection and fused silica capillary column (CP-Sil 88 of 60 m x 0.25 mm). Fatty acids were identified according to the retention times, and were quantified by means of area normalization (%). Macronutrients (Ca, Na, Mg) and micronutrients (Fe, Mn, Mo, Co, Cr, Cu, Zn, and Ni) were determined by following the methodology of the U.S. Environmental Protection Agency - EPA (2007), making use of induced plasma optical emission spectrometry, as recommended by the manufacturer (Vista MPX ICP-OES®, Varian Analytical Instruments, Mulgrave, Australia). K was determined according to the EPA (1974), using atomic absorption spectrometry (SpectrAA 220®, Varian Analytical Instruments, Mulgrave, Australia). For the determination of Se, the technique of hydride generation atomic absorption spectrometry - HGAAS - was used (VGA-77®, Varian Analytical Instruments, Mulgrave, Australia), as seen in EPA (1994).

Semen collection and evaluation

Semen collections were carried out weekly using the artificial vagina method. Data from P1 were considered for the animal distribution into groups and the collections from P5 to P9 were used for comparing the effect of the diets on semen quality, making up a total of 20 consecutive collection weeks and 173 ejaculates per analysis. Immediately after each collection, analyses were done on the aspect, color, pH, and volume of the ejaculate (ml), sperm concentration (x10^6 sptz/ml), gross motility (0 to 5), vigor (0 to 5), and progressive sperm motility (0 to 100%; Adeel *et al*., 2009). Spermatozoa plasma membrane integrity was determined after the preparation of the smears stained with eosin-nigrosin, and the samples were analyzed under optical microscope at 1,000X magnification (Khan and Ijaz, 2008; Iqbal *et al*., 2010, Pal *et al*., 2014). A total of two hundred sperm cells per sample were counted and classified. Sperm that did not uptake the stain (white) were counted as membrane-intact, whereas sperm with any detectable eosin (pink) were counted as membrane-damaged. The percentage of plasma membrane integrity was determined for each slide as the percentage of unstained sperm in the total of sperm counted. For sperm morphology evaluation, the humid chamber technique was used, by means of phase contrast microscopy at 1,000X magnification. The structural defects of the spermatozoa were classified into major defects, minor defects, and total defects (Blom, 1973). Two hundred cells per sample were counted and classified, and results were expressed as percentages.

Experimental design and statistical analysis

The experimental design adopted was random blocks, with three treatments (concentrates), two blocks (age of the animals), and five repetitions (animals) per treatment. First, the data were submitted to the normality test, and the data that did not yield normal distribution underwent logarithmic transformation. The results were submitted to analysis of variance by means of the command PROC GLM of the software Statistical Analysis System, version 6, 1993, and the T-test was used to compare the means (P < 0.05). The data were analyzed considering repeated measures over the period, establishing a relation among dietary treatment, time of semen collection, and their interactions. When there was no interaction due to the collection period, data were submitted to polynomial regression analysis. Moreover, Pearson correlations (P < 0.05) were established for data regarding semen quality and individual nutrient intake, gathered two periods prior to the semen collection, in order to verify the effect of the dietary supplement on the ejaculate characteristics, after the period of time required for two complete spermatogenesis (Sharma and Gupta, 1980). The level of significance adopted was 5%.

Results

The daily dry matter intake of Control animals showed a quadratic behavior (Fig. 1), with interaction between dietary treatment and period of evaluation (P < 0.01), with the average throughout the period of 4.778 ± 1.233 kg of DM. As for the animals in the PKC-Base group, dry matter intake showed a linear behavior, which increased throughout the period and the average over the entire period reached 4.558 ± 1.077 kg of DM. In the CM-Base group, dry matter intake remained steady throughout the period, without regression effect (P > 0.05), and showed a lower value of 3.112 ± 0.693 kg of DM per animal.

$$Y_{control} = 1.721 + 1.321x - 0.113x^2 \ (r^2 = 0.376; \ P = 0.0001)$$
$$Y_{CM\text{-}Base} = 3.464 - 0.192x - 0.019x^2 \ (r^2 = 0.026; \ P = 0.9939)$$
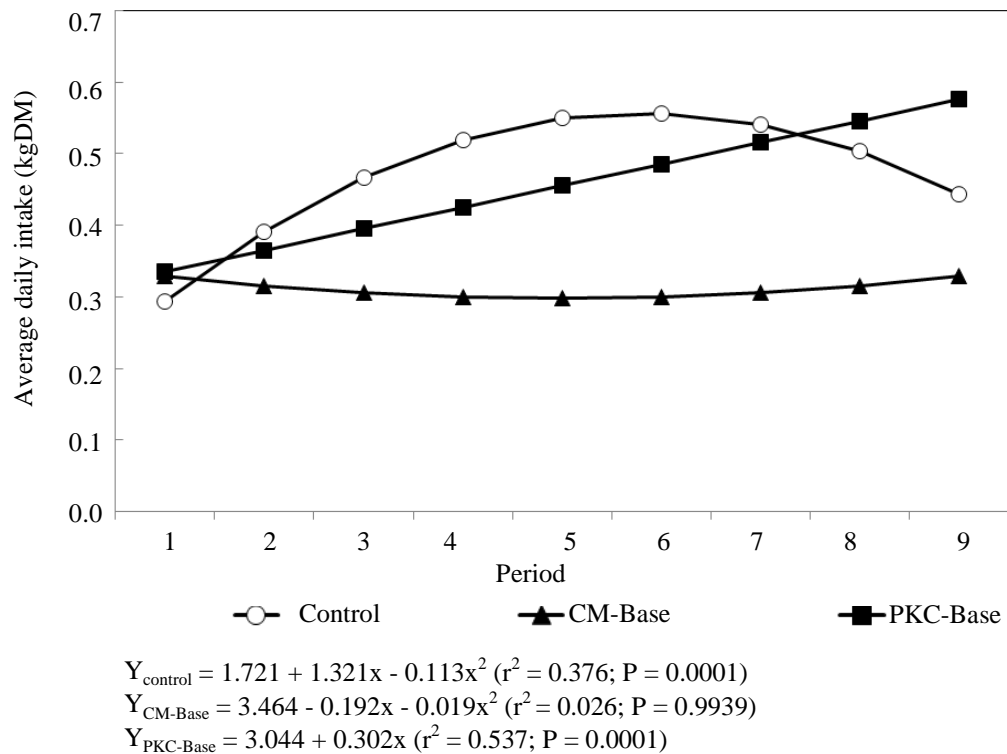$$Y_{PKC\text{-}Base} = 3.044 + 0.302x \ (r^2 = 0.537; \ P = 0.0001)$$

Figure 1. Regression of experimental concentrate intake (kg DM/day) by water buffalo bulls (each period represents a 28-day interval).

Higher crude protein intake was observed in the Control and PKC-Base groups, in comparison to CM-Base animals (respectively 882.2 ± 229.0; 816.0 ± 205.1, and 566.7 ± 127.2 g/day, $P < 0.05$). Lipid intake averages were higher in PKC-Base animals, followed by CM-Base and Control. They were, respectively, 538.7 ± 128.3 g/day, 276.0 ± 62.9 g/day, and 174.0 ± 45.2 g/day ($P < 0.05$).

The intake averages of Ca, Na, and Mg, and of Fe, Mn, Mo, Co, Cr, Cu, Zn, and Se (Table 2) were influenced by a higher content of these elements in the PKC-Base concentrate (data not shown), besides a higher daily intake of this concentrate by the animals. Zn intake was more evident in the PKC-Base and Control groups. In the latter, there was also a higher K intake.

Table 2. Consumption of macro (g/day) and micronutrients (mg/day; means $\pm$ SD) per adult water buffalo raised on pasture with supplemental feeding.

Intake	Concentrate[*]		
	Control	CM-Base	PKC-Base
Macronutrients (g/day)			
Calcium	15.87 ± 4.12^B	8.97 ± 2.01^C	22.24 ± 5.30^A
Sodium	0.62 ± 0.16^B	0.61 ± 0.14^B	1.41 ± 0.34^A
Magnesium	9.84 ± 2.55^B	7.27 ± 1.63^C	11.23 ± 2.67^A
Potassium	43.68 ± 11.34^A	32.37 ± 7.27^B	32.86 ± 7.83^B
Micronutrients (mg/day)			
Iron	783.12 ± 203.29^B	652.33 ± 146.47^C	$1{,}545.14 \pm 368.11^A$
Manganese	103.20 ± 26.79^C	147.57 ± 33.13^B	383.98 ± 91.48^A
Molybdenum	0.14 ± 0.04^B	0.09 ± 0.02^B	5.60 ± 1.34^A
Cobalt	95.46 ± 24.78^C	102.65 ± 23.04^B	126.03 ± 30.03^A
Chrome	7.17 ± 1.87^B	4.30 ± 0.97^C	20.75 ± 4.94^A
Copper	155.43 ± 40.35^B	123.14 ± 27.65^C	188.56 ± 44.92^A
Zinc	202.97 ± 52.69^A	136.19 ± 30.58^B	187.96 ± 44.78^A
Selenium	1.37 ± 0.35^B	1.15 ± 0.26^C	2.80 ± 0.66^A
Nickel	ND	ND	ND

[A,B]Means in a row with different superscripts are different ($P < 0.05$) - T-test; [*]Control (based on ground corn and soybean meal), CM-Base (based on coconut meal), and PKC-Base (based on palm kernel cake); ND: not detected.

88

Anim. Reprod., v.11, n.2, p.85-95, Apr./Jun. 2014

Regarding semen parameters (Table 3), the evaluations were performed after P5, i.e., 112 days after the beginning of supplementation in bulls. The characteristics of volume, gross motility, vigor, and level of morphologic defects of the ejaculates were not influenced by the diets and remained similar (P < 0.05).

Table 3. Effects of concentrate supplements based on industrial by-products on water buffalo semen characteristics (means ± SE).

Semen characteristic	Group[*]		
	Control	CM-Base	PKC-Base
Milky aspect (%)	74.67[A]	72.41[A]	57.97[B]
White color (%)	64.00[B]	86.21[A]	62.32%[B]
pH (0-14)	6.9 ± 0.4[A]	7.0 ± 0.6[A]	6.8 ± 0.5[A]
Volume (ml)	3.6 ± 1.9[A]	3.0 ± 2.1[A]	3.5 ± 1.8[A]
Sperm concentration (x10^6 sptz/ml)	1,326.3 ± 893.8[AB]	1,698.1 ± 1,023.0[A]	1,003.2 ± 569.0[B]
Gross motility (%)	2.9 ± 1.9[A]	3.4 ± 1.8[A]	3.1 ± 1.5[A]
Sperm vigor (0-5)	3.6 ± 1.0[A]	3.7 ± 1.1[A]	3.9 ± 1.0[A]
Progressive sperm motility (%)	59.3 ± 20.5[B]	56.7 ± 24.8[B]	71.7 ± 15.1[A]
Plasma membrane integrity (%)	68.0 ± 19.5[B]	72.0 ± 22.6[B]	82.1 ± 12.2[A]
Major sperm defects (%)	29.6 ± 18.9[A]	27.8 ± 15.3[A]	30.1 ± 21.4[A]
Minor sperm defects (%)	14.6 ± 7.8[A]	13.8 ± 7.1[A]	14.0 ± 7.6[A]
Total sperm defects (%)	44.2 ± 18.5[A]	41.3 ± 16.1[A]	44.3 ± 19.2[A]

[A,B]Means in a row with different superscripts are different (P < 0.05) - T-test; [*]Control (based on ground corn and soybean meal), CM-Base (based on coconut meal), and PKC-Base (based on palm kernel cake).

Considering the data from P5 to P9, progressive sperm motility in PKC-Base animals was always higher than in the animals from the other groups (P < 0.05) and the spermatozoa plasma membrane integrity levels were close to or higher than 80% (Fig. 2). The plasma membrane integrity levels in PKC-Base animals were higher than those observed in the Control and CM-Base groups (P < 0.05), with over 20% more cells with membrane integrity, thus Control animals were adopted as reference.

Protein intake (Table 4) was positively correlated to progressive sperm motility. Regarding fatty acids profiles (%) in the experimental concentrates, higher ratios of polyunsaturated fatty acids were detected in the concentrate supplied to the Control (57.21%) when compared to CM-Base (5.76%) and PKC-Base (6.22%; P < 0.05) groups. Concerning saturated fatty acids, higher ratios were seen in PKC-Base animals (85.76%), followed by CM-Base (58.26%) and Control (20.26%; P < 0.05) groups. Higher levels of ω6 were detected in the Control group (P < 0.05) and the saturated/unsaturated fatty acids ratio was higher in the groups that had received experimental concentrates (Control: 0.25; CM-Base: 1.39; PKC-Base: 6.02; P < 0.05). Higher total levels of unsaturated fatty acids were seen in the Control group (Control: 79.74%; CM-Base: 41.75%; PKC-Base: 14.24%; P < 0.05).
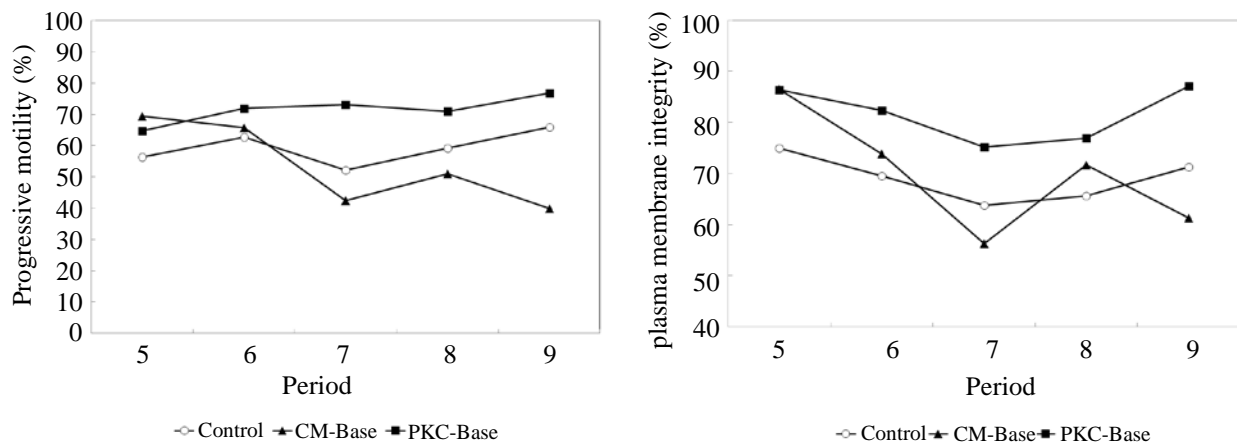

Figure 2. Progressive sperm motility (%) and plasma membrane integrity (%) in spermatozoa of water buffalo bulls supplemented with experimental concentrates.

Table 4. Correlations between nutrient intake, progressive sperm motility, and plasma membrane integrity of water buffalo semen.

Intake	Progressive sperm motility	Plasma membrane integrity
Crude protein	0.21**	NS
Lipids	0.34***	0.36***
Calcium	0.34***	0.24**
Sodium	0.36***	0.34***
Magnesium	0.30***	0.17**
Potassium	0.36***	NS
Iron	0.37***	0.33***
Manganese	0.34***	0.35***
Molybdenum	0.32***	NS
Cobalt	0.38***	NS
Chrome	0.35***	0.33***
Copper	0.33***	0.22**
Zinc	0.17*	NS
Selenium	0.37***	0.33***

NS: non-significant ($P > 0.05$); *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

The intake of macronutrients, such as calcium, sodium, and magnesium, had a positive effect on sperm motility and on plasma membrane integrity. The intake of selenium, iron, cobalt, chromium, copper, manganese, molybdenum, and zinc was also positively correlated with progressive sperm motility, with emphasis on cobalt. Plasma membrane integrity was positively correlated with the dietary levels of iron, manganese, chromium, copper, and selenium.

Discussion

The lower dry matter intake observed in the CM-Base group may be related to the high content of coconut meal in the composition of the experimental feeds, since the increase in coconut meal content in the concentrate, from 0 to 75%, linearly decreases the voluntary intake in sheep (Faturi *et al*., 2010). A previous report indicates that the concentrate intake in water buffalo heifers fed 0.6% BW in diets composed of corn, soybean meal, palm kernel cake, or coconut meal did not vary when the coconut meal content in the concentrate was only 25% (Souza Filho, 2011), which showed that high contents of coconut meal can reduce consumption. PKC-Base intake increased in the periods evaluated, similarly to a previous report regarding water buffalo steers, when palm kernel cake was used in the diet to replace forage, with a higher inclusion level of 60% (Barbosa *et al*., 2010). However, intake results differ from previous data that report a decrease in voluntary concentrate intake when 29.7% palm kernel cake was used in the concentrate (Rodrigues Filho *et al*., 1996). Higher crude protein intake in the Control and PKC-Base groups can be explained due to differences observed in dry matter intake, since the diets had been formulated to be isoproteic. Similar crude protein intake (5.78 to 8.53 g/kg$^{0.75}$, equivalent to 683 to 1,008 g/day for 580 kg BW animals) has been reported, with a content of 20 and 60% palm kernel cake in the diet for buffalo bulls (Barbosa *et al*., 2010). Lipid intake was higher in the PKC-Base and CM-Base groups resulting from higher ether extract content in the palm kernel cake and in the coconut meal compared to the corn meal.

Starting semen evaluations at P5 considered the interval needed to allow spermatogenesis, maturation, and transport of at least one new generation of spermatozoa (Gholami *et al*., 2010) and enabled checking possible nutritional effects on semen quality. The semen's milky aspect was predominant in all experimental groups. However, higher occurrence percentages were found in the Control and CM-Base groups. The white color prevailed, being more frequent in the CM-Base group. The findings corroborate previous data (Vale, 2002), according to which water buffalo semen has a color that varies from white to milky white. The pH of the ejaculates ranged between 6.8 and 7.0 with no difference among the groups, also in accordance to previous findings (Sansone *et al*., 2001; Vale, 2002). The volume of the ejaculates in the present study ranged from 2 to 8 ml (Vale *et al*., 2008) and is similar to the average of 3.07 ± 0.17 ml described for the species (Alavi-Shoushtari *et al*., 2009). Lower sperm concentration values were observed in the PKC-Base group, but they did not deviate from the physiological standards from 524.10 ± 20.70 to $1,031.4 \pm 28.70$ x 10^6 sptz/ml (Kumar *et al*., 1993a), or $1,493$ x 10^6 sptz/ml (Pant *et al*., 2003) in 3-4 year-old buffalos' ejaculates, which is similar to the age group of the bulls used in the present experiment. The gross motility and sperm vigor

90

Anim. Reprod., v.11, n.2, p.85-95, Apr./Jun. 2014

found in this study corroborate the reference data (Vale, 2002), which states that values above 3 are considered good for bull ejaculates.

The PKC-Base animals showed higher progressive sperm motility (P < 0.05), with levels matching the previously described ones, between 70 and 80% (Pant *et al*., 2003; Asadpour *et al*., 2008). Control and CM-Base animals showed similar numbers (P > 0.05), which were close to the ones reported: 66.19 ± 0.51% (Nair *et al*., 2006) and 60.8 ± 1.5 to 69 ± 4.0% (Kumar *et al*., 1993b) for water buffalo bulls. Considering data from P5 to P9, progressive sperm motility in PKC-Base animals was always higher than in the animals from the other groups (P < 0.05) and the levels of plasma membrane integrity of the spermatozoa were close to or higher than 80%, which is considered a positive fact, even if it refers to raw semen.

The levels of plasma membrane integrity in PKC-Base animals were higher than those observed in the Control and CM-Base groups (P < 0.05), with an increase above 20% in the total number of cells with membrane integrity, adopting the Control animals as reference. This protective effect is possibly related to a higher total intake of lipids in PKC-Base animals, as well as to the distinct fatty acid profile of the diet composed of palm kernel cake concentrate, which is rich in ether extracts. It is known that dietary lipid supplementation significantly increases the serum concentration of total lipids, cholesterol and HDL in water buffalo bulls, even after ruminal biohydrogenation of fatty acids (Silva *et al*., 2014). Higher serum levels of cholesterol contribute to increase the concentration of cholesterol on seminal plasma, which is responsible for reducing the premature sperm capacitation, inhibiting rearrangements of phospholipids on sperm membranes and the acrosome reaction (Travis and Kopf, 2002). Since the levels of lipids detected in blood and seminal plasma, including cholesterol, are related to the bovine bull fertility (Beer-Ljubic *et al*., 2009), increments on seminal features of buffalos fed with higher fatty acids levels were expected. Moreover, the increase in the lipid supply benefits the spermatogonia during the initial phase of gametogenesis and is required in the processes of meiosis and differentiation of spermatocytes (Schenk and Hoeger, 2010). Therefore, the lipidaemia has positive effects on sperm morphology and a positive association with the spermatic viability (Silva *et al*., 2014), corroborating the observed data for PKC-Base animals.

Considering the dietary lipid profile, the best levels of plasma membrane integrity were surprisingly achieved with the diet richest in saturated fatty acids. That is unexpected since the diets rich in polyunsaturated fatty acids have become known for raising semen quality in different species, such as in bovine bulls (Gholami *et al*., 2010), rams (Samadian *et al*., 2010; Selvaraju *et al*., 2012), rabbits (Mourvaki *et al*., 2010), and stallions (Brinsko *et al*., 2005). However,

bull semen is rich in polyunsaturated fatty acids (Nair *et al*., 2006). This high content of unsaturated lipids found in the ejaculate can turn the spermatozoa into an excellent target for lipid peroxidation (Brouwers and Gadella, 2003), exposing them to high concentrations of reactive oxygen species and their negative effects, such as adenosine triphosphate depletion, a block in sperm-egg fusion, decreased sperm motility, and irreversible damages to the DNA. The membrane integrity findings in PKC-Base animals were higher than the levels from 74.00 ± 3.04% (Nair *et al*., 2006) to 80.2 ± 3.9% (Rasul *et al*., 2001). This effect can be considered positive, since plasma membrane integrity is a prerequisite for the occurrence of physiological events related to the fertilization process, which include sperm capacitation, binding to the zona pellucida, acrosome reaction, and fusion of gametes (Arruda *et al*., 2005). Although statistically lower than what was observed in the PKC-Base group, the plasma membrane integrity levels in Control and CM-Base animals were close to the reported levels, which ranged from 68.7 ± 2.0% to 75.6 ± 2.1% (Koonjaenak *et al*., 2007a).

Abnormal morphology results were higher than reported levels of 14.1 ± 0.8% (Koonjaenak *et al*., 2007b) and of 15.9 ± 4.7% (Asadpour *et al*., 2008). However, the sperm defect levels were not different in the Control, CM-Base, and PKC-Base groups, which allow inferring that this fact is not related to the addition of coconut meal or palm kernel cake to the animals' diets. In fact, the environment, the semen processing techniques, human error, and time of reaction constitute factors that can influence sperm defect levels.

The correlation observed between protein intake and progressive sperm motility corroborates previous findings (Rekwot *et al*., 1988) that evaluated higher (14.45%) and lower (8.51%) crude protein contents in the concentrate, and identified a significant increase in semen volume, sperm motility, and sperm concentration in bulls supplemented with a higher protein-based diet. Higher lipid intake levels were correlated with increased sperm motility and plasma membrane integrity, confirming the effects of the diet on semen composition and sperm quality, which had been previously reported (Robinson *et al*., 2006). For this reason, higher levels of lipids ingested by the animals in the PKC-Base group can explain the higher progressive motility observed in the ejaculates, as well as the higher level of sperm membrane integrity and stability, which are very important factors to increase the fertility of bulls intended to natural service and to shift the efficiency of the cellular cryopreservation processes in bulls housed in artificial insemination centers.

Despite the higher total levels of unsaturated fatty acids in the Control group, some very important semen parameters for fertilization, such as sperm motility and plasma membrane integrity, curiously improved in PKC-Base animals, regardless of the

importance of the polyunsaturated fatty acids in the diet to provide increased fluidity in the plasma membrane of the spermatozoa (Wathes *et al*., 2007). However, the data obtained are similar to others already reported (Adeel *et al*., 2009), which do not indicate the effect of diets rich in ω3, found in sunflower oil, on sperm motility and plasma membrane integrity of *in natura* semen of water buffalo bulls.

The diet of animals subjected to intensive farming methods has been modified with the use of more modern techniques. The ω6/ω3 ratio, which used to be 1/1, has gone through increments in the ω6 rates, since its concentration in conserved forage is usually low. For this reason, animal feeds have been supplemented with fats derived from oleaginous seeds and elements rich in ω3, in order to decrease ω6 and to increase ω3 intake, and to make animal diets healthier (Wathes *et al*., 2007). In the diets rich in ω3, docosahexaeonic acid yields higher levels of plasma membrane integrity in the spermatozoa and increases their motility (Robinson *et al*., 2006). In fact, considering progressive sperm motility and membrane integrity as crucial aspects for fertilization, it can be claimed that semen quality improved in PKC-Base animals, which diet showed an ω6/ω3 ratio close to 1 (1.29). Similarly, previous reports showed that a dietary supplement offered to rams, with ω6/ω3 ratio close to 1 (0.96), increased the levels of sperm concentration, total motility, and progressive motility after six weeks of intake, when compared to an ω6/ω3 ratio of 1.92 (Samadian *et al*., 2010). It is known that the intake of saturated fatty acids has a negative effect on sperm concentration in humans (Attaman *et al*., 2012). It is possible that this relationship also occurs in water buffalos, since a lower sperm concentration was observed exactly in PKC-Base animals, which had a high intake of concentrate rich in saturated fats, and a high ratio of saturated/unsaturated fatty acids.

The intake of macronutrients, such as calcium, sodium, and magnesium, had a positive effect on sperm motility and on plasma membrane integrity. That corroborates previous reports (Kadirvel *et al*., 2009) which describe the important role of calcium in the fertilization process, especially during sperm capacitation, sperm cell hyperactivation, and induced acrosome reaction through the contact with the zona pellucida. Besides calcium, there have been reports stating that magnesium is linked to total motility, to progressive sperm motility, and to sperm viability in water buffalos (Eghbali *et al*., 2010a). This fact was corroborated by the correlations of these elements with sperm motility and plasma membrane integrity found in the present study.

The intake of selenium, iron, cobalt, chromium, copper, manganese, molybdenum, and zinc were also positively correlated with progressive sperm motility, with emphasis on cobalt. It is known that cobalt has a positive effect on the semen of rams (Kendall *et al*.,

2000). In rats, cobalt poor diets lead to a decrease in the levels of B12 vitamin, resulting in a decrease in the sperm motility parameters. The negative effects on spermatogenesis are more evident and less reversible if the cobalt deprivation persists (Watanabe *et al*., 2003). Although zinc is related to progressive motility and sperm viability (Alavi-Shoushtari *et al*., 2009), its correlation with progressive motility was of lesser magnitude, compared to the other microelements studied, even though it was significant.

Plasma membrane integrity was positively correlated to the dietary levels of iron, manganese, chromium, copper, and selenium. Manganese showed the highest correlation. Iron and copper are associated to plasma membrane integrity in the sperm of water-buffalos (Eghbali *et al*., 2008, 2010b). The deficiency in manganese can impair or depress reproductive functions (Singh, 2008), and manganese can be used as an antioxidant element in the protection of the semen of bulls against lipid peroxidation and oxidative stress. Selenium is associated to sperm motility and plasma membrane integrity, while chromium increases semen quality in rats (Anderson and Polansky, 1981), improving semen fertility. These results highlight the importance of micronutrient supplementation for better semen quality of water buffalo bulls and confirm the findings in the specific literature on the positive influence of micronutrients on sperm parameters.

In conclusion, the dietary supplementation with by-products made with coconut meal and palm kernel cake provided nutrients similar to those found in traditional feeds, with a higher supply of lipids and minerals in the palm kernel cake-based concentrates. The bulls fed with the experimental concentrates showed semen quality that allows suggesting the replacement of corn and soybean, without offering risks associated to nutritional factors. The concentrate made of palm kernel cake provided an increase in semen quality, with higher sperm motility and higher levels of spermatozoa with plasma membrane integrity, which can be related to a higher intake of lipids and minerals.

Acknowledgments

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94

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95