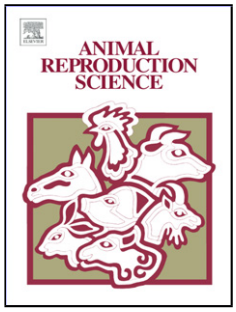

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Effect of dietary supplementation of palm kernel cake on ovarian and hepatic function in buffalo (*Bubalus bubalis*)

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ABSTRACT

To determine the optimal inclusion amount of palm kernel cake (PKC) in a buffalo diet, in the present study there was evaluation of the ovarian activity, metabolism and hepatic function of females that were treated to synchronize the time of ovulation. Twenty-four estrous-cyclic and non-lactating Murrah buffalo with a mean age of 5.7 years were supplemented with 0%, 0.25%, 0.5% and 1% of their body weight (BW) with PKC. Animals were subjected to the Ovsynch protocol (beginning of protocol = D0). The ovaries were examined and the blood was collected on D10 (follicular phase) and D17 (luteal phase). Follicular and luteal development and serum progesterone concentrations were not affected by diet (P>0.05). Serum concentrations of cholesterol were greater in animals supplemented with PKC in amounts at 0.5% of BW or less with PKC, regardless of the phase of the estrous cycles when evaluations occurred (P<0.05). Concentrations of HDL-cholesterol were similar (P>0.05) during the follicular and luteal phases. Triglyceride concentrations increased linearly (P=0.03) as percentage of PKC inclusion diets increased during the follicular phase, but were similar in the luteal phase (60.0 mg/dL; P = 0.51). Amount of PKC supplementation did not affect the concentrations of alanine aminotransferase, but there was a greater amount of aspartate aminotransferase (AST) and gamma glutamyl transferase (GGT) during both phases of the estrous cycle (P<0.05). Animals supplemented at 1.0% of BW with PKC had greater AST and GGT concentrations than what is recommended for buffalo. The results of the present study indicate PKC supplementation of buffalo diets does not affect the development of the ovarian follicle and corpus luteum nor the peripheral concentration of progesterone, even though there are greater serum concentrations of total cholesterol and triglycerides. Because the amount of PKC supplementation in the present study does not result in hepatic dysfunction when fed at the 0.5% of BW amount, it is suggested that this agro-industrial

byproduct of high nutritional value may be a new alternative for dietary supplementation of grazing buffalo.

Keywords: Buffalo; Cholesterol; Liver function; Ovsynch; Estrous synchronization

1. Introduction

Buffalo are very well adapted to environmental conditions in countries having a similar tropical climate with the countries of origin for this species. For this reason, buffalo farming has increased in Brazil, especially in the Brazilian Amazon region where there are about 74% of the number of 1,370,941 buffalo in the country (IBGE, 2016). This species has been farmed worldwide on small and medium sized properties, where it has essential economic and social importance, with a favorable performance for beef and milk production with the different breeding systems that have been developed. Even though there is favorable adaptation to different environments, buffalo have a lesser reproductive efficiency than cattle, mainly due to a longer period from birth to puberty and prolonged calving intervals (Zicarelli, 2010), which is probably related to inadequate genetic selection and/or poor management (Vale, 2000), mainly due to thermal stress resulting from the high temperatures and humidity in areas where there are buffalo used for dairy and meat production (Marai and Haeeb, 2010).

Dietary fat supplementation has been used for cattle (Thatcher and Staples, 2000; Funston, 2004; Lopes et al., 2009, 2011) and buffalo (Malik et al., 2011; Nazir et al., 2013) as a strategy to increase reproductive performance by considering the influence of nutrition and dietary energy. One of the main effects of dietary energy supplementation on the reproductive efficiency of ruminant females is due to the action of fatty acids, which can cause metabolic

and endocrine changes that affect ovarian function (Dias et al., 2009). Lipid supplementation in cattle increases the functional capacity of the ovaries, prolongs the corpus luteum lifespan and increases plasma concentrations of progesterone, which has a positive effect on reproductive function (Staples et al., 1998; Mcnamara et al., 2003) and embryonic quality (Cerri et al., 2009). Even though there are beneficial effects in female cattle, few studies have been conducted to evaluate the effects of fatty acid supplementation on the reproduction of female buffalo.

Dietary fatty acid supplementation can increase plasma progesterone concentrations because of several factors, such as the following: greater supply of cholesterol (progesterone precursor) in tissues and body fluids, including the ovaries and corpus luteum (Stronge et al., 2005; Demetrio et al., 2007), increased synthesis of this hormone (Grummer and Carroll, 1991) and even a decreased catabolism rate due to reduced hepatic metabolism (Hawkins et al., 1995). The maintenance of relatively greater plasma concentrations of progesterone may be important in buffalo, because one of the main causes of early embryonic mortality is the insufficient production of this hormone (Campanile et al., 2005, 2007, 2008).

Regardless of the benefits of proper nutrition, the use of animals for food production is costly especially when there is use of grains that can be alternatively used in human diets. In tropical conditions, some commercially grown tree species produce significant amounts of agro-industrial byproducts. These byproducts have low added value and, therefore, it is important to conduct research on the nutritional value of these byproducts when included in diets of ruminants (Rodrigues Filho et al., 1993; Abdalla et al., 2008). The use of a byproduct with desirable nutritional constituent's results in a reduction in costs for feed, improves the capacity to use animal in production agriculture and minimizes the effects of season of the year on food production for ruminants. The oil palm (*Elaeis guineensis* Jacq.) byproduct is

available throughout the year and when used as a dietary supplement results in efficient product production by food producing animals in Brazil with about 1.68 million tons produced annually (IBGE, 2017). For every 100 kg of fruits processed for mechanical extraction of oil, there is production of about 3 kg of palm kernel cake (PKC) (Costa et al., 2011), an abundant agro-industrial byproduct that can be used in livestock feeds. In addition, the PKC, also known as *dendê* almond cake, has desirable nutritional characteristics such as percentage crude protein (14%), digestibility (60%) and ethereal extract (12.0%) that make this byproduct attractive for use in animal feeds (Rodrigues Filho et al., 2001).

The objective of the present study was to evaluate the effect of PKC supplementation on the ovarian functions of buffalo when there were treatments to induce ovulation synchronization using the Ovsynch protocol. Furthermore, there were assessment of dietary supplementation with PKC on plasma concentrations of progesterone, lipoproteins, triglycerides and liver enzymes. Considering the importance of buffalo farming in tropical regions, there is potential for use of PKC in the diets of buffalo for improvement of the reproductive efficiency of the species.

2. Materials and methods

2.1. Experimental site

The experiment was performed at the Emprapa's Experimental Station for Buffalo Farming (Belém, PA, Brazil, $1^{0}26'$S and $48^{0}24'$W) from May to October 2015. This region's climatic type is Afi (Köppen), with high indexes of temperature, insolation, rainfall and relative humidity, with respective annual averages of 26.7 °C, 2,338 hours, 3,001 mm and 84% with a less rainy period from June to November (Bastos et al., 2002).

2.2. Experimental diets and animals

In conducting the study, there was use of 24 estrous cyclic, non-lactating healthy Murrah buffalo with a mean age of 5.67 ± 1.60 years and mean weight of 684.2 ± 62.8 kg which is similar to these values for buffalo used in previous studies (Láu, 1999; 2006). These buffalo were randomly assigned to treatment groups that were balanced in terms of animal age, weight and order of calving. All procedures that were used were approved by the Ethics Committee on Animal Use (CEUA), São Paulo State University (UNESP), Campus Jaboticabal (protocol no. 5128/15).

The buffalo were maintained in paddocks containing *Urochloa brizantha* cv. Marandu that are utilized in an intermittent grazing system at the experiment station. The experimental area of 10 hectares was subdivided into eight paddocks where the animals grazed in groups to avoid the undesirable effects of pasture quality on study outcomes. Mineral salt and clean water were supplied *ad libitum* in covered troughs and artificial drinking fountains, respectively.

There were four treatments with daily amounts of PKC fed being: 0%, 0.25%, 0.5%, and 1% of the animal's body weight (BW), based on dry matter. Wheat bran was added at 0.15% of the animal's BW to all dietary treatments to increase the palatability of PKC. The animals were fed the PKC supplement for 60 days to adapt to the diet and, subsequently, the dietary supplementation was provided for 120 days (experimental phase). Supplementation was provided in individual troughs in the feeding shed once every morning. The PKC that was not consumed (refused feed) were collected daily to determine amount of PKC consumption. The quantity of PKC supplied to the buffalo was adjusted monthly based on body weight of buffalo at beginning of the month the study was initiated.

2.3. Chemical analysis

The PKC samples were collected monthly and frozen at -20 ºC for subsequent analysis for contents of dry matter (DM; method INCT-CA G-003/1), organic matter (OM; INCT-CA M-001/1), crude protein (CP; method INCT-CA N-001/1), ethereal extract (EE; INCT-CA G-005/1), neutral detergent fiber corrected for ash and protein (NDFap; INCT-CA F-002/1, INCT-CA M-002/1 and INCT-CA N-004/1) and acid detergent fiber corrected for ash and protein (ADFap; INCT-CA F-004/1, INCT-CA M-003/1 and INCT-CA N-005/1), using the methods recommended by the National Institute of Science and Technology in Animal Science (INCT-CA) (Detmann et al., 2012). For the analyses, the samples were thawed at room temperature and ground in a Willey type mill with a 1 mm sieve. The samples from feed that was fed each month were combined and the percentages of chemical composition for dietary components are shown in Table 1.

The lipid fraction of PKC was extracted using the Butt or Soxhlet method (AOCS, 2009). The PKC fatty acid composition (Table 2) was quantified using a capillary gas chromatograph (CGC Agilent 68650 series GC system, AGILENT, USA). The chromatographic procedures included the use of a capillary column DB 23 Agilent (50% cyanopropyl) – methylpolysiloxane, measuring 60 m, with an internal diameter of 0.25 mm and 0.25 μm of film. The chromatograph operating conditions were as follows: column flow, 1 mL/min; linear speed, 24 cm/s; detector temperature, 280 ºC; injector temperature, 250 ºC; oven temperature, 110 ºC, maintained for 5 min; heating from 110 to 215 ºC (5ºC/min), maintained at 215 ºC for 24 min; drag gas, helium; volume injected, 1.0 μL.

2.4. Stage of estrous cycle synchronization and ultrasonic evaluation of ovaries

During the phase of adaptation to dietary supplementation, the ovarian structures of buffalo were evaluated by transrectal ultrasonography (US) using a 7.5 MHz linear transducer (Scanner DP-3300Vet®, Shenzhen Mindray Bio-medical Electronics Co. Ltd., China) to assess the effectiveness of the estrous synchronization hormonal regimen on the estrous cyclicity in the animals by evaluating whether there was presence of a corpus luteum and absence of indications that there were dysfunctions of the ovaries and uterus as a result of treatments. After the adaptation period, on a random day of the estrous cycle, there was imposing on all buffalo a regimen of hormonal treatment to induce synchronization of time of ovulation using the Ovsynch protocol, which is similar to the one developed for cattle (Pursley et al., 1995), but adapted for buffalo (Neglia et al., 2003). The treatment was as follows: on the first day of the synchronization protocol (Day 0), the buffalo were treated with buserelin acetate, a GnRH analog (Syncroforte® 20 μg im; Ourofino Animal Health, Brazil); on Day 7, there was treatment with cloprostenol sodium (Clocio® 500 μg im; BIMEDA Brasil S.A., Brazil); and on Day 9, there was treatment with buserelin acetate (Sincroforte® 20 μg im). The estrous synchronization protocol was repeated in the experimental animals four times at 30-day intervals during the experimental period, as depicted in Figure 1.

During the experimental phase, the diameters of the preovulatory follicle, corpus luteum and ipsilateral ovary were measured on days D10 (follicular phase) and D17 (luteal phase) from the time of initiation of the hormonal treatment regimen for estrous synchronization, always by the same operator. At each evaluation of the ovaries, the measurements of the ovarian and uterine structures were conducted through assessment of "frozen" images on the screen. In the case of a cavitary corpus luteum, the area of the luteal cavity was subtracted from the area of the corpus luteum to determine the area of luteal tissue.

2.5. Blood collection and assays

Blood samples were collected on days D10 and D17 after the beginning of the ovulation synchronization protocol. The jugular vein was punctured with a 21 G needle (Labor Import Comercial Imp. Exp. Ltd., Brazil) for vacuum blood collection in two sterile glass tubes (Vacutainer, 10 mL, Becton Dickinson, USA), one without anticoagulant and the other with clot activator. The tubes were centrifuged (Quimis centrifuge, Brazil) at 1500 X *g* for 10 minutes at room temperature. Serum fraction samples were placed in microtubes in triplicate. The microtubes were kept frozen at -20 ºC for subsequent analysis of metabolites and hormones using the single thawing method.

Serum concentrations of total cholesterol, HDL-cholesterol fraction, triglycerides and the enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma glutamyl transferase (GGT) were determined by using colorimetric enzymatic reactions that were performed using commercial kits (Labtest Diagnóstica S.A., Brazil), according to the manufacturer's recommendations and the methods described by Allain et al. (1974) and Fossati and Lorenzo (1982). The measurements were performed on an automatic biochemical analyzer (BS 120®, Shenzhen Mindray Bio-medical Electronics Co., Ltd., China).

Progesterone concentrations were quantified by radioimmunoassay (RIA) using the blood samples collected on D17 after the beginning of the ovulation synchronization protocol. Commercial kits (MP Biomedicals, California, USA) were used according to the manufacturer's recommendations. All samples were analyzed by conducting procedures at the same time to reduce procedural variability. The minimum detectable amount of progesterone was 0.187 ng/mL. The coefficients of intra-assay and inter-assay variations were 6.95% and 8.67%, respectively.

2.6. Statistical analysis

Statistical analyses were performed using the Statistical Analysis System for Windows, SAS Institute, USA (SAS 9.1, 2003). The experimental design was completely randomized, with four treatments and six replications with repeated measurements (four periods of synchronization). The data were assessed for normality (Proc Univariate, Shapiro-Wilk test) to determine if there was a normal distribution. Results from use of an analysis of variance (ANOVA), where the effects of percentage of PKC supplementation (diet effect), estrous synchronization period (replication effect) and interaction among factors were tested, indicated there were no effects of replication on the estrous synchronization results and the interaction was also not significant. The polynomial regression analysis was used to analyze the effect of the percentage of PKC supplementation. Parametric variables were expressed as mean and standard error of the mean (mean ± SEM). The level of significance was considered to be $P < 0.05$.

3. Results

Although there is a relationship between increased dietary lipid inclusion and reduced intake, this effect was not a factor in the present study until the inclusion amount of 0.5% of BW with PKC (Table 1). This outcome was probably a function of the relative amount of the ethereal extract that was a maximum of 5.6% (Table 3), which is less than the recommended upper limit (6% PV) for ruminants (Palmquist and Mattos, 2006).

There was only a refusal to consume the PKC in the 1% BW group, with an average daily consumption of 0.7% BW in this group (Table 3). There was a quadratic reduction in the percentage of supplement consumption in the 0.5% BW treatment group ($P<0.05$). The

reduction in CMS (kg/day) can be attributed, in part, to the increase in ethereal extract contents in the diets that was observed when there was the greatest inclusion in the diet (1.0% of BW) of the PKC (Table 3).

The different percentages of PKC supplementation did not affect (P>0.05) the diameters of the preovulatory follicles, corpora lutea and ovaries (Table 4). This was determined by the ultrasonic evaluations performed on D10 and D17 after the beginning of synchronization at a time that animals were in the follicular and luteal phases of the estrous cycle, respectively. The mean diameter of the preovulatory follicle was 13.34 ± 0.29 mm and that of the ipsilateral ovary was 22.19 ± 0.33 mm. In the luteal phase, the mean diameter of the corpus luteum was 15.88 ± 0.33 mm and that of the ipsilateral ovary was 22.75 ± 0.45 mm.

Serum concentrations of total cholesterol increased gradually and there was an association of serum concentrations with the percentage of PKC provided in the diet. Accordingly, there was an increasing quadratic (P = 0.0151) and linear (P = 0.0589) effect during the follicular and luteal cycles of the estrous cycle, respectively (Figure 2).

Serum concentrations of HDL-cholesterol were not affected by the increased percentage of dietary PKC (Table 5), with mean blood serum concentrations of 54.86 ± 1.08 mg/dL in the follicular phase (P = 0.0841) and 53.73 ± 1.00 mg/dL in the luteal phase (P = 0.4695) of the estrous cycle.

For serum triglyceride concentrations, there was an increasing linear effect (P = 0.0339) related to the amount of PKC in the diet during the follicular phase (Figure 3). There was no effect, however, during the luteal phase (P = 0.5100) when there was similarity among the treatment groups, with a mean value of 59.99 ± 2.12 mg/dL (Table 5).

Serum concentrations of progesterone in the luteal phase (D17) were not affected by amount of dietary PKC (Table 5), with mean values being 8.31 ± 0.63 ng/mL.

Liver function assessments indicated there was no change in serum concentrations of ALT among treatment groups, neither during the follicular (P = 0.5472) nor in luteal (P = 0.7656) phases of the estrous cycle, with mean values of 31.40 ± 0.99 IU/L and 31.25 ± 1.09 IU/L, respectively (Table 5). The AST enzyme activity was affected (P<0.001) by the increased amount of dietary PKC with an increasing linear effect in the follicular and luteal phases (Figure 4) of the estrous cycle. A similar effect occurred with GGT enzyme activity, which was affected by the percentage of PKC in the diet (Figure 4), with an increasing linear effect both in the follicular (P<0.05) and luteal (P<0.001) phases of the estrous cycle.

4. Discussion

There is not consistent thought on the efficacy of dietary fat supplementation for ruminants because although fatty acid supplementation has been suggested to improve reproductive performance in cattle (Santos et al., 2008), results of other studies indicate that the feeding of diets containing saturated or polyunsaturated fatty acids did not improve the number of follicles or corpora lutea in dairy cows where there was super-stimulation of ovarian follicular development for purposes of superovulation (Thangavelu et al., 2007) or beef heifers (Childs et al., 2008a). Similar to what was reported in these previous studies, in the present study, there was no effect of supplementation with PKC on follicular and luteal development of estrous synchronized non-lactating buffalo. Even though there is a lack of effect of dietary supplementation on the development of ovarian structures, the results indicate the need for a greater understanding of the effects of nutrition and dietary fat supplementation on the physiology of reproduction in the buffalo species, because these

animals have greater feed conversion efficiency compared to cattle due to greater ruminal cellulolytic efficiency and greater utilization of poor quality fodder (Tewatia and Bhatia, 1998). Thus, such physiological differences make it difficult to extrapolate the interpretation of results among these different ruminant species.

Results of different studies indicate there is an increased effect (Nazir et al., 2013; Cordeiro et al., 2015) or a lack of effect (Childs et al., 2008b; Malik et al., 2011) of dietary fat supplementation on serum progesterone concentrations. There were also greater concentrations of this steroid in some studies with cattle and it was hypothesized to be because of decreased hepatic metabolism (clearance) resulting from lipid supplementation (Hawkins et al., 1995; Sartori and Mollo, 2007). It, however, is still necessary to perform evaluations where there can be measurements of the hepatic flow function of the animals with fat dietary supplementations. Another explanation for greater steroid concentrations is the possible increase in release of LH pulses (Hightshoe et al., 1991), resulting in an increased diameter of follicles and luteal bodies (Raes et al., 2004). In this present study, however, there was no difference in progesterone concentrations on D17 after the beginning of the estrous synchronization protocol between the group of buffalo in which diets were supplemented with PKC and those that were not supplemented. Regardless of the PKC concentrations used as supplement, there were no changes in follicular and luteal development. Consequently, the absence of variation in diameter, circumference or area of luteal tissue indicates there may not be an effect of dietary supplementations of fatty acids on progesterone biosynthesis, even though increased serum concentrations of total cholesterol was observed in treated animals.

Cholesterol had a significant effect on ovarian physiology because it is the precursor of the steroid hormones secreted by this organ. In the present study, regardless of the estrous cycle phase evaluated, an inclusion of 0.5% of BW with PKC in the diet led to increased

serum concentrations of total cholesterol compared to non-treated animals. The greatest values in the present study, however, were consistent with the reference values (89-134 mg/dL) for buffalo used for milk production (Arshad et al., 2005; Hagawane et al., 2009). The increased gastrointestinal fatty acid uptake due to PKC dietary supplementation for buffalo is believed to result in increased serum concentrations of total cholesterol via synthesis and accumulation of cholesterol and cholesterol esters in blood (Hawkins et al., 1995; Stronge et al., 2005; Demetrio et al., 2007). In the present study, the results were similar to those reported previously (Nazir et al., 2013; Silva et al., 2014) where there was also assessment of the possibility of modulating cholesterol plasma concentration by increasing the ethereal extract in diets. It is interesting to note that a supplementation of PKC at 1.0% of BW resulted in a reduction in serum concentrations of total cholesterol in the follicular and luteal phases. The mechanism for this is not fully understood.

There is no effect on the HDL-cholesterol fraction between buffalo where there was not or was dietary supplementation of PKC in the different stages of the estrous cycle. The HDL concentrations were less than those reported by Campanile et al. (2010) where there were mean values of 64 mg/dL in buffalo supplemented with a corn-based diet.

In the present study, the hepatic function of the animals was also evaluated to determine the adequate proportions of PKC (in % BW) that can be supplemented in the diet without causing damage to or metabolic alterations. Dietary supplementation with PKC did not alter ALT activity in buffalo where there was a mean value (31.33 IU/L) that was less than the mean value of 42.8 IU/L reported by Nazir et al. (2013) in buffalo supplemented with linseed.

Buffalo supplemented with PKC, however, had greater serum concentrations of AST liver enzyme during the two phases of the estrous cycle evaluated in the present study with

there being greater concentrations as inclusions rates of PKC in the diet increased. The greater concentrations of this liver enzyme with supplementations of PKC is probably because the liver has a fundamental function in the homeostasis of fat metabolism by removing excess plasma lipoproteins after intestinal absorption or by returning lipid-bound fatty acids in the form of triglycerides, phospholipids and cholesterol esters linked to lipoproteins to the blood (Niemeyer, 1978). Nevertheless, in the present study, no hepatic dysfunction was observed with the inclusion of PKC at as much as 0.5% of BW, as the AST enzyme activity had a mean value (129.06 IU/L) similar to the value of 131.5 IU/L that was reported by Gandra et al. (2011), but less than that of 143.1 IU/L reported by Gomes et al. (2010), which is considered the reference value for Murrah buffalo. Supplementation at 1.0% of the BW with PKC in the present study resulted in an increase in the AST enzyme activity with the mean value being greater than the reference value for buffalo (167.47 compared with 143.1 IU/L), as described by Gomes et al. (2010). This finding indicates there is a possible hepatic dysfunction as a result of feeding the PKC in the present study, which should be avoided when there are dietary fat supplementations.

Independent of the evaluated phases (follicular or luteal) of the estrous cycle in the present study, mean serum concentrations of GGT enzyme in buffalo supplemented with as much as 0.5% of BW with PKC was similar to the reference value reported by Gomes et al. (2010) for the same species and breed (31.83 IU/L compared with 33.3 IU/L). There, however, was a significant increase in the mean value (38.7 IU/L) of this enzyme activity in buffalo treated with the 1% of BW with PKC, which may result in undesirable effects on the hepatic function of the animals and, therefore, on nutrient absorption capacity.

5. Conclusions

The results of the present study indicate that PKC supplementation for buffalo does not affect the development of the ovarian follicle and corpus luteum nor the peripheral concentration of progesterone, even though there is a greater concentration of total cholesterol and triglycerides. Because there was not hepatic dysfunction when PKC was supplemented in amounts as great as 0.5% of the BW, we can suggest that this agro-industrial byproduct of high nutritional value may be a new alternative for dietary supplementation of grazing buffalo.

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Conflict of interest

The authors have no conflict of interest to disclose.

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Figure Legends

Fig. 1. Depiction of experimental design and hormonal treatment regimen for the ovulation synchronization protocol (Ovsynch) used in the experiment

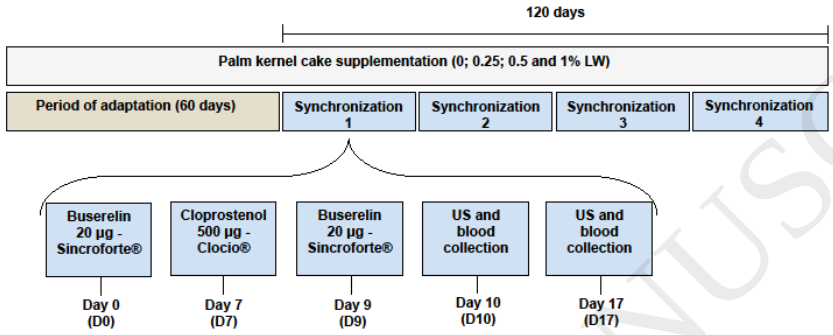

Fig. 2. Serum concentration of total cholesterol in buffalo supplemented with different percentages of palm kernel cake (PKC) based on body weight in the follicular (A) and luteal (B) phases of an estrous cycle in which synchrony of ovarian follicular development among animals was induced

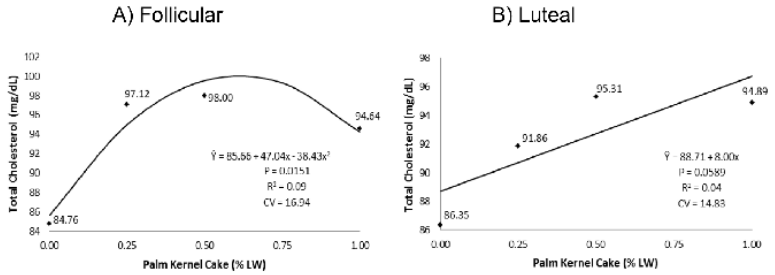

Fig. 3. Serum concentrations of triglycerides during the follicular phase of estrous synchronized buffalo in which there was dietary supplementation with different percentages of palm kernel cake (PKC) based on body weight

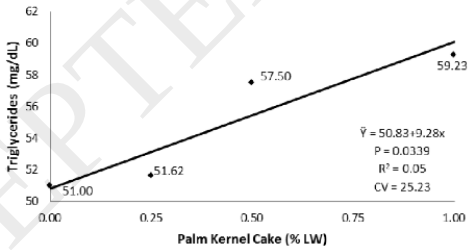

Fig. 4. Serum concentrations of AST and GGT enzymes in buffalo supplemented with different percentages of palm kernel cake (PKC) based on body weight in the follicular (A

and B, respectively) and luteal (C and D, respectively) phases of an estrous cycle in which synchrony of ovarian follicular development among animals was induced

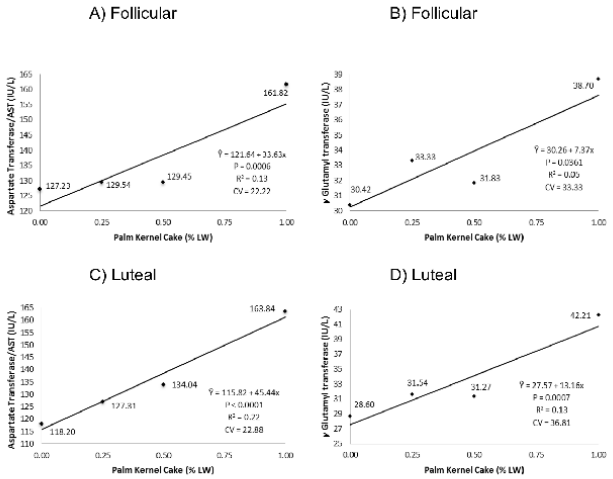

Table 1 Chemical compositions of palm kernel cake (PKC), wheat bran, grass (*Urochloa brizantha cv. Marandu*) and diets supplied to the buffalo

Chemical composition	Ingredient		
	PKC	Wheat bran	Grass
Dry matter (g/kg)	955.30	890.10	295.00
Crude protein (g/kg DM)	102.80	158.90	85.20
NDFap (g/kg DM)	530.30	434.00	676.70
ADFap (g/kg DM)	350.00	137.40	393.80
Organic matter (g/kg DM)	962.00	945.50	934.00
Ethereal extract (g/kg DM)	102.20	33.30	25.90

Components	PKC supplementation (% BW)			
	0.00	0.25	0.50	1.00
DM (g/kg)	133.52	372.34	611.17	1088.82
CP (g/kg DM)	23.84	49.535	75.24	126.64
NDFap (g/kg DM)	65.10	197.67	330.25	595.40
ADFap (g/kg DM)	20.61	108.11	195.61	370.61

	Organic matter (g/kg DM)	141.83	382.32	622.83	1103.83
	Ethereal extract (g/kg DM)	5.00	30.54	56.10	107.20

Abbreviations: DM: Dry matter; BW: Body weight; NDFap: Neutral Detergent Fiber corrected for ash and protein; ADFap: Acid Detergent Fiber corrected for ash and protein. Wheat bran was added as 0.15% BW in all treatments

Table 2 Fatty acid composition of palm kernel cake (PKC)

Fatty acids	% mass/mass
Saturated	82.40
C6: 0 (Caproic)	0.7
C8: 0 (Caprylic)	3.47
C10:0 (Capric)	3.28
C12:0 (Lauric)	46.58
C14:0 (Myristic)	16.41
C15:0 (Pentadecanoic)	0.06
C16:0 (Palmitic)	8.57
C17:0 (Margaric)	0.13
C18:0 (Stearic)	2.69
C20:0 (Arachidic)	0.16
C22:0 (Behenic)	0.11
C24:0 (Lignoceric)	0.17
Unsaturated	17.60
C18: 1 (Oleic)	15.06
C18: 2 (Linoleic)	2.36

C20: 1 (Eicosenoic)	0.18
Total	100.00
Unsaturated/saturated ratio	0.21

Table 3 Amounts fed, where there were refusals to eat and consumption of palm kernel cake (PKC) on a dry matter basis of buffalo supplemented with different percentages of PKC based on body weight

	PKC supplementation (% BW)				
	0.00	0.25	0.50	1.00	*P* value
Supply kg DM	26,55 ±3,16	72,68 ±5,677	116,25±9,59	206,53±20,43	<,0001
Leftovers kg DM	0	0	0	61,12 ±5,57	<,0001
Consumption % BW	100,0	100,0	100,0	70,39	<,0001

Abbreviations: DM: Dry matter; BW: Body weight. Wheat bran was added as 0.15% of BW in all treatments.

Table 4 Measurements of ovaries and ovarian structures (follicles and corpora lutea) during the follicular (D10) and luteal (D17) phases in buffalo where there was dietary supplementation with different percentages of palm kernel cake (PKC) based on body weight

Ovarian measurements	PKC supplementation (% BW)				*P*-value
	0.00	0.25	0.50	1.00	
Follicular phase					
FolDiam[1]	13.17 ± 0.63	14.00 ± 0.65	12.90 ± 0.42	13.21 ± 0.59	0.7046
FolCirc[2]	38.91 ± 1.91	41.00 ± 2.01	37.94 ± 1.28	38.56 ± 1.60	0.5998
FolArea[3]	124.89 ± 11.79	139.40 ± 12.50	115.75 ± 7.55	119.47 ± 8.78	0.4122
DiamOv[4]	21.93 ± 0.69	22.09 ± 0.69	22.72 ± 0.64	21.93 ± 0.60	0.6991
Luteal phase					
CLDiam[5]	16.34 ± 0.75	16.25 ± 0.77	15.68 ± 0.55	15.19 ± 0.56	0.1860
CLCirc[6]	48.07 ± 2.26	48.33 ± 2.23	46.33 ± 1.70	45.58 ± 1.79	0.3030
CLArea[7]	188.76 ± 18.24	192.34 ± 18.85	173.53 ± 12.77	169.15 ± 13.06	0.2924
DiamOv[4]	22.56 ± 0.80	23.85 ± 0.93	22.19 ± 0.95	22.31 ± 0.88	0.5312

Measurements are presented as mean ± standard deviation.

Abbreviations: BW: Body weight; [1]FolDiam: follicular diameter; [2]FolCirc: follicular circumference; [3]FolArea: follicular area; [4]DiamOv: ovarian diameter; [5]CLDiam: corpus luteum diameter; [6]CLCirc: corpus luteum circumference; [7]CLArea: corpus luteum area.

Table 5 Concentrations of blood metabolites during the follicular (D10) and luteal (D17) phases in buffalo where there was dietary supplementation with different percentages of palm kernel cake (PKC) based on body weight

Metabolites	PKC supplementation (% BW)				*P* value
	0.00	0.25	0.50	1.00	
Follicular phase					
HDL[1] (mg/dL)	51.29 ± 2.15	54.92 ± 2.38	58.04 ± 2.17	54.71 ± 1.26	0.0841
ALT[2] (IU/L)	31.71 ± 2.36	29.29 ± 2.19	32.50 ± 1.41	32.41 ± 1.91	0.5472
Luteal phase					
HDL[1] (mg/dL)	51.95 ± 1.75	53.23 ± 2.06	55.77 ± 2.55	53.84 ± 1.19	0.4695
ALT[2] (IU/L)	31.90 ± 2.50	30.50 ± 2.22	32.09 ± 1.81	30.47 ± 2.30	0.7656
Trig[3] (mg/dL)	57.65 ± 4.90	57.00 ± 5.03	64.91 ± 2.70	60.21 ± 4.03	0.5100
P_4[4] (ng/mL)	7.40 ± 0.90	8.75 ± 1.34	7.25 ± 0.90	9.97 ± 1.76	0.2324

Measurements are presented as mean ± standard deviation.

Abbreviations: BW: Body weight; [1]HDL: HDL-cholesterol, [2]ALT: alanine aminotransferase, [3]Trig: triglycerides, [4]P_4: progesterone.