EFFECTS OF SUPPLEMENTATION WITH PROTECTED POLYUNSATURATED FATTY ACIDS ON PRODUCTIVE AND HORMONAL PARAMETERS OF EMBRYO RECIPIENT HEIFERS[1]

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ABSTRACT: Supplementation with protected polyunsaturated fatty acids (PPUFA) has positive effects on cow reproduction. Therefore, the aim of this study was to evaluate the effects of adding a source of PPUFA to energy supplements for embryo recipient heifers on productive performance and plasma concentrations of progesterone, cholesterol and insulin. For this purpose, 44 Angus x Hereford embryo recipient heifers (average body weight = 385 kg) raised on pasture were studied in a completely randomized design. The effects of PPUFA added to isocaloric energy supplements for 60 days on production parameters and serum concentrations of cholesterol, progesterone and insulin were evaluated. The treatments consisted of individual supplementation with: 1) control (no supplement); 2) corn (corn, 70%; soybean meal, 30%); 3) PPUFA supplement (Megalac-E®, 30%; soybean meal, 20%; commercial ration, 50%). The treatments did not affect (P>0.05) dry matter intake, pregnancy rates, or serum insulin concentration. However, PPUFA supplement increased (P<0.05) serum cholesterol and progesterone concentrations when compared to the other treatments. Despite the lack of a difference (P>0.05) in dry matter intake between treatments, PPUFA supplement increased (P<0.05) average daily gain compared to the control and corn treatments. The inclusion of PPUFA in energy supplements offered to heifers used in an embryo transfer program increased average daily gain and serum concentrations of cholesterol and progesterone, but did not affect pregnancy rates.

Keywords: embryo recipients, supplementation, protected polyunsaturated fatty acids, cholesterol, progesterone.

EFEITOS DA SUPLEMENTAÇÃO COM ÁCIDOS GRAXOS POLIINSATURADOS PROTEGIDOS SOBRE OS PARÂMETROS PRODUTIVOS E HORMONAIS DE NOVILHAS RECEPTORAS DE EMBRIÕES

RESUMO: A suplementação com ácidos graxos poliinsaturados protegidos (AGPIP) tem efeitos positivos sobre a reprodução de fêmeas bovinas. O objetivo deste estudo foi analisar os efeitos da adição de uma fonte de AGPIP em suplementos energéticos para novilhas receptoras de embriões e avaliar seu efeito sobre o desempenho produtivo e concentrações plasmáticas de progesterona, colesterol e insulina**. F**oram utilizadas quarenta e quatro novilhas Angus x Hereford receptoras de embriões (peso médio = 385 kg) em pastagem, em um delineamento experimental completamente casualizado. Foi avaliado o efeito de 60 dias de suplementação com ácidos graxos poliinsaturados protegidos da degradação ruminal dentro de suplementos energéticos isocalóricos sobre parâmetros produtivos e concentrações plasmáticas de colesterol, progesterona e insulina. Os tratamentos foram: 1) Controle (sem suplemento), 2) Milho (milho, 70%; farelo de soja, 30%), 3) AGPIP (Megalac-E®, 30%; farelo de soja, 20%; ração comercial, 50%). O tratamento não afetou

(P>0,05) a taxa de prenhes e a concentração plasmática de insulina. No entanto, a suplementação com AGPIP aumentou (P<0,05) as concentrações plasmáticas de colesterol e progesterona, quando comparadas aos demais tratamentos. Apesar de não haver diferença (P>0,05) no consumo de matéria seca entre os tratamentos, o suplemento AGPIP aumentou (P<0,05) o ganho médio diário quando comparado aos tratamentos controle e milho. A incorporação de ácidos graxos poliinsaturados protegidos em suplementos energéticos fornecidos a novilhas utilizadas em um programa de transferência de embriões aumentou o ganho de peso médio diário e as concentrações de colesterol e progesterona, mas não afetou as taxas de prenhes.

Palavras-chave: receptoras de embrião, suplementação, ácidos graxos poliinsaturados protegidos, colesterol, progesterona.

INTRODUCTION

Embryo recipient heifers play an important role in the success or failure of embryo transfer. The maintenance of pregnancy in recipient heifers is one of the main challenges of successful embryo transfer. Some studies have shown positive effects of fatty acids on embryo development (Thangavelu *et al*., 2007; Childs *et al*., 2008; Petit *et al*., 2008; Cerri *et al*., 2009) and pregnancy rates (Lopes *et al*., 2007; Lopes *et al*., 2009), which are also positively correlated with higher cholesterol (Takahashi *et al*., 2013; Cordeiro *et al*., 2015) and progesterone concentrations (McNeil *et al*., 2006; Hess *et al*., 2008; Lopes *et al*., 2009; Peres *et al*., 2009; Salas-Raso *et al*., 2011.; Cordeiro *et al*., 2015).

These findings may be explained by the fact that fats are precursors for the synthesis of cholesterol and steroid hormones (Staples *et al*., 1998; Leroy *et al*., 2014). Studies have demonstrated higher concentrations of androstenedione and estradiol in preovulatory follicles of cows fed diets rich in C18:1n-9 and C18:2n-6 (Zachut *et al*., 2008) and increased LH pulsatility in cattle fed polyunsaturated fatty acids (PUFA) (Hightshoe *et al*., 1991). Furthermore, the diameter of the dominant preovulatory follicle was found to be increased in cows fed diets rich in C18:2n-6 (Robinson *et al*., 2002) or C18:3n-3 (Ambrose *et al*., 2006). According to Funston (2004), the increase in preovulatory follicle size may be due to elevated plasma concentrations of LH, a hormone that stimulates the final stages of follicular growth, with a consequent increase in the size of the preovulatory follicle and subsequent greater corpus luteum (Raes *et al*., 2004), or to a decrease in hepatic metabolism of this hormones (Hawkins *et al*., 1995).

In view of the above considerations, our hypothesis was that supplementation with protected polyunsaturated fatty acids (PPUFA) increases the plasma concentrations of cholesterol and progesterone without affecting plasma insulin concentration, which could improve reproductive responses in embryo transfer programs. The aim of this study was to evaluate the effect of adding a source of PPUFA to energy supplements for embryo recipient heifers on productive performance and plasma concentrations of progesterone, cholesterol and insulin.

MATERIALS AND METHODS

The experiment was conducted in southwestern Rio Grande do Sul, Brazil. Animals were cared for in accordance with the Animal Care Committee of the School of Agronomy, Universidade Federal do Rio Grande do Sul, RS, Brazil. The experimental area consisted of improved native pasture oversewn with white clover (*Trifolium repens*) and ryegrass (*Lolium multiflorum* Lam.) in a 50-hectare area, which was rested for 50 days prior to the start of the experiment.

Forty-four Angus x Hereford heifers [2 years old, body weight = 384 ± 36 kg; body condition score higher than 3 (scale 1 to 5)] were randomly assigned to the different supplement treatments. All animals were housed in the same paddock. The period of supplementation lasted 60 days, starting 30 days before embryo transfer. Heifers were transferred to individual pens daily at 7:00 am to receive the supplement. Average daily gain (ADG) was determined as the average amount of weight gained per day over a given period of time the animal has been on supplement. For this purpose, the body weight of heifers was measured at intervals of 30 days after a 12-hour fast.

The treatments were: 1) control (no supplement); 2) corn (corn, 70%; soybean meal, 30%); 3) PPUFA (Megalac-E®, 30%; soybean meal, 20%; commercial ration, 50%). The components of the supplements

and pasture and the fatty acid profile of Megalac-E® are shown in Tables 1, 2, and 3.

Forage availability (kg dry matter, DM/ha) (Table 1) was determined every 30 days by taking 7 samples/ha of pasture measuring 0.25 m^2, followed by drying in an oven (65°C). Additionally, samples were collected every 28 days for the evaluation of forage quality using simulated grazing (Johnson, 1978) and were analyzed for crude protein (CP), total digestive nutrients, neutral detergent fiber, acid detergent fiber, and lignin (AOAC, 1995) (Table 1). Total digestive nutrients were determined using the equation described by Weiss (1999).

Estrus synchronization was performed by administering two doses of prostaglandin ($PGF_2\alpha$) at an interval of 11 days between the first and second dose. Starting 34 hours after the second $PGF_2\alpha$ dose, estrus activity was monitored at 7:00 am and 7:00 pm over 4 days.

Seven days after estrus detection, the corpus luteum was scored by a veterinarian experienced in animal reproduction and 30 embryo recipients were selected. The embryos used for the experiment were frozen embryos from two Angus donor cows. The distribution of the embryos per treatment was random and the embryos were deposited in the uterine horn ipsilateral to the corpus luteum. Pregnancy was confirmed 30 days after embryo transfer by ultrasonography.

On the day of embryo transfer and at confirmation of pregnancy, blood samples were collected from the coccygeal vein into a vacutainer tube without anticoagulant. The serum concentrations of progesterone, insulin and cholesterol were determined by radioimmunoassay using commercial kits (DPC Medlab, São Paulo, Brazil, for progesterone, and MP Biomedical for insulin). Serum cholesterol was determined by spectrophotometry (Sommers *et al*., 1975).

Data were analyzed in a completely randomized design with three treatments. The animal was the experimental unit. Initial weight, weight on the day of embryo transfer, final weight, and weight gain were analyzed with a linear model using the general ANOVA procedure (Statistix, 1995). Serum concentrations of cholesterol, progesterone and insulin were evaluated using the general ANOVA procedure, with weight at embryo transfer and at conformation of pregnancy being fitted as covariates. Means were compared using the Tukey test at a significance level of 5%. Pregnancy rates were analyzed using the chi-square test (Statistix, 1995). All variables showed a normal distribution and homogeneous variances. The statistical model for initial weight, weight on the day of transfer, final weight and ADG was: $Y_{ij} = \mu + T_i + \varepsilon_{ij}$, where Y_{ij} is the j^{th} observation associated with the i^{th} treatment (T); μ is the mean; T_i is the effect of the i^{th} treatment (i=1,2,3), and ε_{ij} is the j^{th} error related to the ij^{th} observation. The model for serum concentrations of insulin, cholesterol and progesterone was: $Y_{ij} = \mu + T_i + B_jP_j + \varepsilon_{ij}$, where Y_{ij} is the j^{th} observation associated with the i^{th} treatment (T); μ is the mean; T_i is the effect of the i^{th} treatment (i=1,2,3); B_j is the regression coefficient associated with the covariate body weight (day of transfer and/or pregnancy confirmation) of the j^{th} animal (j=1,2…, 44), and ε_{ij} is the j^{th} error related to the ij^{th} observation.

Table 1. Forage availability (kg DM/ha) and chemical composition of the supplement and pasture ingredients

Variable[1]	Ingredients of the supplements				Pasture		
	Commercial ration	Soybean meal	Corn	Megalac-E®[2]	Day 0	Day 30	Day 60
kg DM/ha					3,937	2,267	1,976
CP (% DM)	13.4	26.2	9.6	0.4	10.2	9.3	7.8
EE (% DM)	8.4	6.3	5.5	44.2	3.4	2.5	2.5
Ashes (% DM)	11.5	32.2	3.8	24.4			
NDF (% DM)	44.7	5.1	50.6		65.0	60.8	61.3
ADF (% DM)	5.7	0.9	3.1		30.4	31.8	31.8
Lignin (% DM)	2.3	82.5	0.9		4.4	5.6	8.5
TDN (% DM)	77.8	80.7	80.9	106	61.7	58.2	59.3

[1]DM: dry matter; CP: crude protein; EE: ether extract; NDF: neutral detergent fiber, ADF: acid detergent fiber; TDN: total digestible nutrients. [2]The product is sold in packages of 25 kg and the minimum guaranteed levels are 85% fat (as fatty acids; % DM) and 7.5% calcium (% DM). Megalac-E® contains about 42% linoleic acid (omega 6) and 3% linolenic acid (omega 3).

Table 2. Percentage of ingredients and chemical composition of the supplements

Components[1]	Supplement	
	PPUFA	Corn
Megalac-E (%)	30	0
Corn (%)	0	70
Soybean meal (%)	20	30
Commercial ration (%)	50	0
	Chemical composition	
DM (%)	83	80.46
CP (% DM)	21.65	19.32
EE (% DM)	21.48	6.41
TDN (% DM)	86.45	85.52

[1]DM: dry matter; CP: crude protein; EE: ether extract; TDN: total digestible nutrients.

Table 3. Fatty acid profile of protected polyunsaturated fatty acids (Megalac-E®)

Fatty acid profile	%
Lauric acid (12:0)	0.1
Myristic acid (14:0)	0.2
Palmitic acid (16:0)	17.5
Palmitoleic acid (16:1)	0.3
Stearic acid (18:0)	5.1
Oleic acid (18:1)	31.7
Linoleic acid (18:2)	39.8
Linolenic acid (18:3)	2.7
Others	2.6

RESULTS AND DISCUSSION

Although supplementation increased (P<0.05) blood concentrations of cholesterol and progesterone, no effects on pregnancy rates were detected in the present study (P>0.05; Table 4). However, these data need to be evaluated with caution because of the small number of animals used in this experiment. According to Chapman and Seidel (2008), a large number of animals (n = 496) is needed to detect a 10% difference in pregnancy rates between two treatments with a power of 0.90.

Lopes *et al*. (2009) supplemented 435 nulliparous and multiparous lactating crossbred *Bos indicus* cows with PPUFA from the end of estrus synchronization (d 7) until fixed-time embryo transfer (d 28) and found higher pregnancy rates (P=0.07) for cows supplemented with PPUFA compared to control cows (49.6 vs. 37.7%). Similar results have been reported in another study by these authors (Lopes *et al*., 2007) who observed higher pregnancy rates in Nelore heifers that received PPUFA compared to control.

Furthermore, feeding fat can be a good strategy to stimulate follicular growth and steroid production (Leroy *et al*., 2014). In this experiment, heifers receiving PPUFA supplement had higher serum cholesterol concentrations on the day of embryo transfer compared to the corn and control treatments (P<0.05; Table 4). These results agree with other studies investigating lipid supplementation of cows (Lammoglia *et al*., 1996) and sunflower seed supplementation for timed artificial insemination and timed embryo transfer (Cordeiro *et al*., 2015). In line with these studies, in heifers fed dried distiller grains with solubles, which contain relatively high fat concentrations, higher total plasma cholesterol was observed when compared to the low-fat diet and control (Anderson *et al*., 2015). In addition, cows supplemented with PPUFA exhibited 21% higher cholesterol concentration than controls (Carrol *et al*., 1992).

Takahashi *et al*. (2013) investigated the effects of feeding PUFA to Japanese Black heifers following embryo transfer to recipient Holstein heifers. The authors observed significantly higher total cholesterol concentration on day 15 to 19 of feeding (after embryo transfer) in the PUFA group (117.4 mg/dl) compared to the control group (95.0 mg/dl). Additionally, the pregnancy rate after embryo transfer was higher for cows fed PUFA. These findings might be explained by the fact that fats are precursors for the synthesis of cholesterol and steroid hormones (Thomas *et al*., 1997; Staples *et al*., 1998), or by alterations in prostaglandin synthesis (Petit and Twagiramungu, 2006; Childs *et al*., 2008).

In addition to increasing cholesterol concentration, fat supplementation has been shown to increase plasma progesterone concentrations (Ryan *et al*., 1992; Hawkins *et al*., 1995; Lopes *et al*., 2009; Salas-Raso *et al*., 2011; Cordeiro *et al*., 2015). This has been hypothesized to be related to increased plasma cholesterol concentrations, the main precursor for steroid synthesis (Staples 1998). Studies have also shown that higher circulating progesterone concentrations are positively associated with conception rates in beef and dairy cattle (McNeil *et al*., 2006; Hess *et al*., 2008; Peres *et al*., 2009). In the present study, heifers fed the PPUFA supplement had higher (P<0.05; Table 4) progesterone concentrations at the time of embryo transfer

Table 4. Mean ± standard deviation of serum progesterone, cholesterol and insulin concentrations and pregnancy rates of heifers receiving the control, corn and PPUFA supplements at the time of embryo transfer and at confirmation of pregnancy

Variable	Supplement		
	Control (n=10)	Corn (n=9)	PPUFA (n=11)
	Cholesterol (mg/dL)		
Embryo transfer	146± 25.37 b	146±25.96 b	171±30.90 a
Confirmation of pregnancy	143±17.54 a	154± 20.04 b	198±36.84 b
	Progesterone (ng/ml)		
Embryo transfer	3.68± 1.17 b	3.65± 1.55b	5.41±1.84a
Confirmation of pregnancy	4.27± 0.56 b	5.77±0.93ab	7.32±1.34 a
	Insulin (μIU/ml)		
Embryo transfer	14.10±4.26	12.52±3.27	13.66±3.82
Confirmation of pregnancy	15.07±7.01	14.21±3.03	14.97±5.24
	Pregnancy rate (%)		
30 days after embryo transfer	36.36 (4/11)	77.77 (7/9)	40 (4/10)

Means followed by different letters differ between treatments by the Tukey test (P<0.05).

than heifers receiving the other treatments. At the time of pregnancy confirmation, progesterone concentrations were higher (P<0.05) in heifers receiving the PPUFA supplement when compared to control but not to corn supplementation. Apparently, corn supplementation during the 30 days after embryo transfer had the same effect as PPUFA on serum progesterone. Other authors (Hawkins *et al*., 1995) suggested a reduction in hepatic metabolism to be the main factor responsible for the increase in circulating progesterone concentrations in cows fed a diet rich in lipids.

Lopes *et al*. (2009) reported higher plasma concentrations of progesterone 7 to 8 days after ovulation (7 days after artificial insemination) in animals supplemented with PPUFA when compared to control, which was also associated with an 11% increase in pregnancy rate. In addition, supplementation of Indubrasil cows with bypass fat during the early postpartum period in the dry tropics had a positive effect on re-initiation of ovarian activity and increased plasma concentrations of cholesterol and progesterone (Salas-Razo *et al*., 2011). However, not all studies found increases in plasma progesterone concentrations when animals are supplemented with fat (linoleic or linolenic acid) (Childs *et al*., 2008; Wamsley *et al*., 2005).

Although the concentrations of cholesterol and progesterone were higher for the PPUFA treatment when compared to control, the type of supplement used did not influence plasma insulin concentration (P>0.05; Table 4). However, diets rich in long-chain fatty acids increase hepatic gluconeogenesis due to increased propionic acid production in the rumen (Keele *et al*., 1989). This increased gluconeogenesis has been associated with increases in plasma concentrations of insulin and insulin-like growth factor I (IGF-I), which are thought to influence the population of ovarian follicles (Thomas and Williams, 1996). Gong *et al*. (2002), studying dairy cows in early lactation, found that the use of diets that increase circulating insulin may advance the first postpartum ovulation and increase the conception rate in the first service. Furthermore, at estrus, IGF-I and cholesterol concentrations and the diameter of the first dominant follicle were higher in cows supplemented with linoleic acid (18: 2, n-6) than in cows that did not receive this supplement (Robinson *et al*., 2002). Nonetheless, Staples *et al*. (1998) collected data from 17 experiments that measured serum insulin concentration in cows supplemented with lipids and reported that 8 papers indicated that insulin concentration decreased in cows supplemented with fat, while the others found no differences in insulin concentration.

The average DM availability during the experimental period was 2,956 kg per ha (Table 1), suggesting no restrictions in voluntary intake. Voluntary intake is probably not influenced by forage availability unless pasture availability is less than 2,000 kg DM/ha (Kahn, 2014). During the experimental period, the pasture was of medium

quality (Table 5) (ELIS *et al*., 1988) and average CP was 9.1%, demonstrating that there was no restriction in ruminal microbial function or intake. These functions would be reduced if the CP concentration were less than 7% (COCHRAN *et al*., 1998). VALADARES *et al*. (1997) supplied diets with different protein levels (7, 9.5, 12 and 14.5% DM) to Zebu steers and found that a CP level of 7% decreased DM and organic matter intake, probably because this concentration of CP was insufficient to promote proper microbial growth. In contrast, CAVALCANTE *et al*. (2005) found that total apparent digestibility of DM, organic matter and CP increased linearly with increasing CP concentration in the diet (10.5, 12, 13.5, and 15% CP of DM).

The weights at the beginning of the experiment did not differ (P>0.05) between treatments (Table 5) and dietary treatment did not affect (P>0.05) DM intake (Table 4). The total ADG of PPUFA heifers was greater (P<0.05) than that of heifers receiving the control and corn supplements. This higher ADG observed in heifers receiving the PPUFA and corn supplements may be explained by the fact that consumption of the supplement supplies additional energy and protein and provides a more optimal rumen environment, improving forage digestibility (SINCLAIR *et al*., 1995). Bodine and Purvis II (2003) reported that offering an energy supplement to steers fed medium quality pasture (7% CP) increased ADG (0.73 kg/animal/day) compared to non-supplemented controls (0.24 kg/animal/day). Heifers fed the PPUFA supplement had higher ADG than those submitted to the corn and control treatments probably because the addition of fat can improve energy density in the diet without increasing carbohydrate intake (SALLA *et al*., 2003). Furthermore, multiple supplements administered at 0.3% of live weight have been shown to promote higher weight gain in finishing crossbred steers during the rainy season in Brazil than that obtained with mineral mixture alone (NASCIMENTO *et al*., 2010).

Table 5. Mean ± standard deviation of body weight, average daily gain (ADG), total intake and dry matter intake (DMI) of heifers receiving the control, corn and PPUFA supplements at the time of embryo transfer and at confirmation of pregnancy

Variables[1]	Supplement		
	Control	Corn	PPUFA
Initial body weight (kg)	392±39,88	361±39,55	376±28,87
	ADG (kg)		
Initial period to embryo transfer	0.80±0.13 c	0.91±0.91 b	1.02±0.093 a
Embryo transfer to final period	0.57±0.08 b	0.64±0.07 b	0.90±0.10 a
	30 days before embryo transfer		
Total supplement intake (kg; 30 days)	0	12.16±1.03	12.60±0.86
DMI of supplement (kg/day)	0	0.38±0.03	0.39±0.03
	30 days after embryo transfer		
Total supplement intake (kg; 30 days)	0	12.75±1.35	13.02±0.41
DMI of supplement (kg/day)	0	0.40±0.01	0.41±0.01

Means followed by different letters differ between treatments by the Tukey test (P<0.05).

CONCLUSION

Supplementation of heifers used in an embryo transfer program with PPUFA as a component of energy supplements increased ADG and plasma concentrations of cholesterol and progesterone, but did not influence pregnancy rates. However, more research using a larger numbers of animals is needed to better evaluate the effects of this supplementation on pregnancy rates.

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