

The study of different levels of RDP in the ration of lactating cows and their effects on estradiol and progesterone levels in the blood

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Abstract

In an effort to sustain milk production during early lactation, dairy producers often feed protein in excess of requirements and recommendations. Unfortunately, overfeeding protein may be associated with a decline in fertility. The detrimental effect of excess protein has been linked to the ruminally degradable fraction (rumen-degradable protein, RDP) of dietary protein. Ruminally degradable protein or ruminally undegradable protein in excess of requirement can contribute to reduced fertility in lactating cows. Dietary protein nutrition or utilization and the associated effects on ovarian or uterine physiology have been monitored with urea nitrogen in blood or milk. Twenty-one multiparous Holstein cows in the late of lactation period were used in complete randomized design to determine the effect of excessive intake of ruminally degradable crude protein on the some reproductive hormones (estradiol and progesterone levels in the blood). Experimental periods were 6 wk in length, with d 1 to 14 used for adjustment and wk 2 and wk 6 used for a sampling (blood, and milk). Three concentration of a rumen-degradable protein (RDP) supplement according to National Research Council recommendations (9.3, 11.4, and 14% of dry matter intake) were treatments. No significant effect of concentration of RDP supplement was detected on difference levels of both hormones from week 2 to week 6. Higher undegradable protein or escape protein showed highest value for increasing progesterone levels but simultaneously lowest value for estradiol. In this regard no significant correlation was observed between estradiol with plasma urea nitrogen (PUN) ($P = -0.167$) and progesterone with PUN ($P = 0.002$), but significant correlation (0.51) was observed between estradiol and progesterone ($P = 0.0123$).

Key words: Degradable intake protein, estradiol, progesterone, blood urea nitrogen

Introduction

Reproductive management concepts need to be integrated into the nutritional and environmental management programs of dairy cows. High milk yield and good reproductive performance are important to the profitability of a dairy enterprise. However, the association between milk yield and reproduction is antagonistic (Butler and Smith, 1989). High milk yield during early lactation retards development of ovarian follicles, prolonging the postpartum interval to first ovulation; high yield also is antagonistic to the expression of estrus and is associated with reduced conception rates. Nutrient requirements must be satisfied from dietary sources and mobilization of body reserves. During early lactation, the rate of increase in milk yield exceeds that of DMI, resulting in a negative energy balance. Diets containing 18 to 19% CP are required in early lactation to support high milk yield (NRC, 1989). However, excessive RDP (>63% of total CP) may depress fertility (Canfield et al., 1990). Providing supplemental RUP may enhance reproduction 1) by improving energy balance by decreasing excessive ruminal ammonia that is energetically costly to excrete or 2) by lowering protein degradability, thereby reducing concentrations of ammonia, urea, or other nitrogenous compounds that are toxic to ova, sperm, or embryos (Sklan and Tinsky, 1993). Blood urea nitrogen (BUN) concentration is variable and is effected by rumen degradable protein intake, undegradable protein intake, energy intake, water intake, liver function and elimination in the urine.

The main objectives of this study were to determine the effect of supplemental undegradable protein in different proportion of concentration in total mixed ration (TMR), on two main reproductive hormone (estradiol and progesterone).

Materials and Methods

Twenty-one multiparous Holstein cows in the late of lactation period were used in complete randomized design to investigate the effect of rumen degradable protein on two main reproductive hormone (estradiol and progesterone). Experimental periods were 6 wk in length, with d 1 to 14 used for adjustment and week 2 and week 6 used for a blood and milk sampling. Three concentration of a rumen-degradable protein (RDP) supplement according to National Research Council recommendations, 1989 (9.3, 11.4, and 14% of dry matter intake) were treatments. The ratio of RDP/UDP in the ration of A, B and C were 50:50, 65:35 and 80:20 respectively (Table 1).

Table 1. Ingredients and nutrient composition (% of DM) of experimental diets.

	Rations		
	A	B	C
Dietary components			
Alfalfa hay, long	17	20	25
Corn silage	30	25	20
Barley grain	3	11	32
Corn grain	15.5	15.5	13.5
Beet pulp	15	1.5	2
Cotton seed meal	.5	20	2
Soybean meal	10.5	1	.5
Fish meal	4	.5	.2
Wheat bran	2	3	1
Urea	-	.2	1.5
Dicalcium phosphate	1.05	.86	.85
Limestone	.5	.5	.5
Salt	.7	.7	.7
Sodium bicarbonate	.25	.25	.25
Nutrient composition			
CP ¹	18.4	18.3	18.3
RDP ¹	9.4	11.4	13.9
UDP ¹	9	6.9	4.4
ADF ¹	19.2	19.4	17.5
eNDF ²	30	33	28
NFC ²	33	31	42
TDN ¹	70.7	70.4	69.7
NE _L (Mcal/kg) ¹	1.62	1.61	1.60

¹ Calculated according to NRC (1989) for diet component.

² Calculated according to CNCP (2002) for diet component.

On the end of week 2 and week 6, milk and blood samples were taken at 2 h after feeding. Blood samples were taken from the coccygeal artery or vein. Samples were heparinized, held at 4 °C maximum 4h until plasma was prepared, and stored at -25 °C. The testing for urea concentration was involved taking a sample of plasma or milk and using a spectrophotometer to measure the change in color when a reagent was added that acts specifically with urea. Usually this method involves a dye agent, diacetylmonoxime that reacts with the urea molecule to form a pink color. The intensity of pink correlates with concentration of urea in the fluid. Estradiol and progesterone were measured in the blood plasma by the Enzyme-linked immunosorbent assay (ELISA) method. The general linear model and Pearson correlation procedures of SAS (2003) were used to analyze the data.

Result and discussion

The results of experiment are presented in Table 2. As planned, total N intake were not different among dietary treatments. The increase in dietary RDP as a percentage of CP, was not accompanied linearly for MUN, plasma urea nitrogen (PUN). In this regard, Roseler et al. (1993) and Moharrery (2004) reported, which concentration of urea in blood is proportional to the concentration of urea in milk. Therefore, milk urea N should be a good predictor of urinary N excretion (UN) by dairy cows (Kohn et al., 1997; Jonker et al., 1998; Moharrery, 2004). Formulation of diets with fat supplementation and sources of UIP may influence not only milk production but also the reproductive performance of the herd. Supplemental fat in the diet has often improved pregnancy or conception rates (Staples et al., 1998). Diets that contain excess protein or degradable intake protein (DIP) may have a negative impact on fertility (Ferguson and Chalupa, 1989), but the effect is not well documented.

Table 2. Effect of different levels of RDP in the ration of lactating cows and their effects on nitrogen in plasma and milk, and plasma hormones.

Item	Rations			SEM ¹	P>F ²
	A	B	C		
Milk yield, kg/d	17.27 ^a	13.70 ^b	12.09 ^c	1.0974	.0001
Total N intake, g/d	397.8	404.8	404.2	3.0034	.5813
MUN, mg/dl	15.29 ^b	20.47 ^a	17.87 ^{a b}	.7250	.0131
PUN, mg/dl	16.23 ^c	23.34 ^a	19.04 ^b	.7426	.0001
Estradiol pg/ml	4.36 ^{ab}	4.53 ^a	4.20 ^b	.0877	.0503
Progesterone ng/ml	5.83 ^a	5.96 ^a	5.12 ^b	.2184	.0303

MUN= milk urea nitrogen, PUN= plasma urea nitrogen.

^{a,b,c} Means in rows with no common superscripts are different (P<0.05).

¹SEM= Standard error of the mean.

²Probability of a significant effect of diet.

No significant effect of concentration of RDP supplement was detected on difference levels of both hormones from week 2 to week 6 (P>0.05; data was not showed).

Significant effect of concentration of RDP supplement was detected on the reproductive hormone in the plasma (P<0.05). With increase of RDP in the ration estradiol and progesterone decreased linearly (P<0.05), but to see the effect of RDP on reproductive hormones, the level of RDP should be reach to 80% of the total crude protein in the TMR in lactating dairy cows. Higher undegradable protein or escape protein showed highest value for increasing progesterone levels but simultaneously lowest value for estradiol. In this regard, concentration of PUN showed weak correlated to progesterone (r= 0.002) and weak negatively correlated to estradiol concentration in the plasma (r= -0.167; Figure 1). No significant correlation was found between MUN and both estradiol and progesterone concentration in the blood (P>0.05; Figure 2). But the correlation between two hormones were significant (r= 0.493; P=0.023), which has been shown in the Figure 3.

Across the literature, an excess amount of either RDP or RUP results in lower fertility; consideration of protein fractions, rather than CP, explained much of the variation in conception rate that was observed among the studies (Ferguson and Chalupa, 1989). Because RDP and RUP are metabolized and utilized separately and by different organs in the lactating cow, the common element of their metabolism when in excess of requirement is the formation of urea. Excess of either RDP or RUP increased PUN and altered uterine pH to a similar degree (Elrod et al., 1993). Those observations further support the potential for urea as the common mediator of uterine effects of excess RDP or RUP. In addition, PUN varies inversely with uterine pH (Elrod et al., 1993) and, thereby, is a possible mediator of decreased fertility associated with elevated PUN or MUN (Butler et al., 1996).

In sheep, Burrin et al. (1989) reported that increased feed intake led to increased liver blood flow and liver oxygen consumption. In addition, sows with greater feed intake had greater blood flow in the hepatic portal vein (Symonds and Prime, 1989). Given that the liver is the major site of progesterone and estradiol-17 β metabolism (Parr et al., 1993; Freetly and Ferrell, 1994), it is logical that increases in ammonia in the blood would increased the blood flow in the hepatic portal vein for detoxification of ammonia and consequently greater metabolic clearance rate of these steroids due to an elevation in liver blood flow.

Formulation of diets with fat supplementation and sources of UIP may influence not only milk production but also the reproductive performance of the herd. Supplemental fat in the diet has often improved pregnancy or conception rates (Staples et al., 1998). Diets that contain excess protein or degradable intake protein (DIP) may have a negative impact on fertility (Ferguson and Chalupa, 1989), but the effect is not well documented.

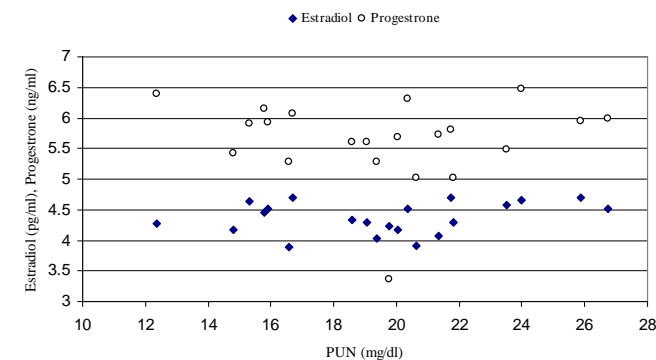


Figure 1. Relationship between plasma urea nitrogen (PUN) and estradiol and progesterone

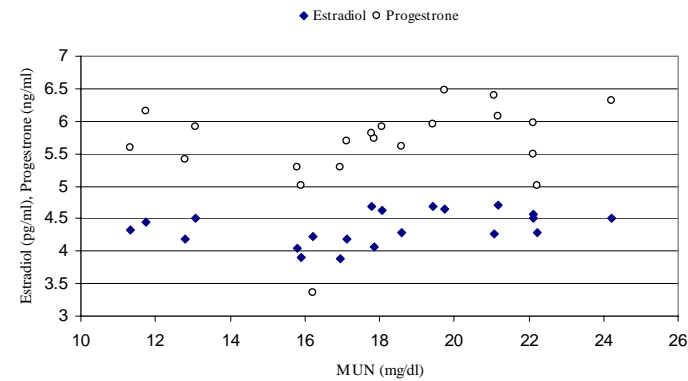


Figure 2. Relationship between milk urea nitrogen (MUN) and estradiol and progesterone

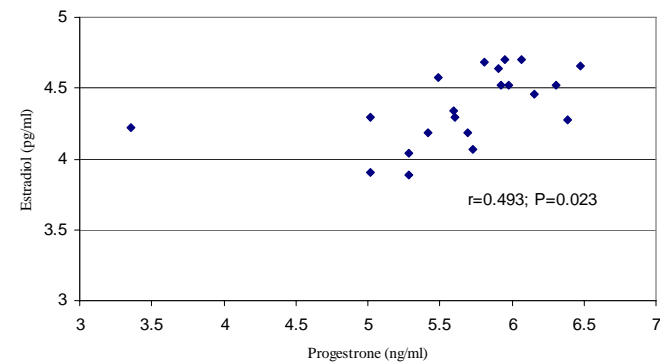


Figure 1. Relationship between estradiol and progesterone

Implications

Feeding excess ruminally degradable protein to dairy cattle can be deleterious to fertility, particularly if there is inadequate energy supplied to the rumen to reduce ammonia concentration. This effect on fertility may be mediated through an alteration in the estradiol and progesterone profile in the plasma, or other reproductive organ environment in which the embryo must grow. Use of a relatively undegradable protein source may allow increased milk production as well as alleviation of the effects on fertility.

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