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Effect of Flushing on Body Weight Changes, Nutrient Digestibility and Litter Size of Nulliparous Does

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Abstract: Nulliparous crossbred does were flushed 1 week before and after mating with four diets containing 18, 20, 22 and 24% CP and placed on 18% CP during pregnancy and lactation. Fecal samples were collected in the second week of flushing for 4 days for proximate analysis. Parameters determined were body weight change and feed intake during flushing, nutrient digestibility, litter size and total milk production. Doe weight gain at end of flushing increased with increase in protein level with does on 24% CP gaining almost twice more (182 g) than those on 18% CP (91.67 g). Doe feed intake was higher before mating and decreased after mating in all the treatments. Protein level did not affect digestibility of dry matter, ash or crude fibre. Crude protein digestibility (85%), digestible crude protein (252.8 g kg^{-1}) and ether extract (26.9) intake were higher with 24% CP compared with the other levels. Litter size at birth and alive at birth indicated a significant quadratic trend with protein level during flushing.

Key words: Rabbits, flushing, protein, intake, weight, digestibility, litter size

INTRODUCTION

Growth and reproductive performance of rabbits depend largely on their ability to digest feed effectively. Their ability to digest feed depends on the nutrient composition of the diet. Protein is important in the nutrition of rabbits. During pregnancy, 20% of digested nitrogen is retained for conceptus growth and doe body tissue accretion while milk yield during lactation is affected by protein level (Partridge and Allan, 1982). Omole (1982) reported a protein range of 18-22% for efficient rabbit production in the tropics.

Improved reproduction has been achieved in farm animals, by increasing feed intake or nutrient content above the maintenance level (flushing), for a short period before mating to increase litter size in pigs (Ashworth, 1991) and ewes (Robinson, 1990; Smith and Somade, 1994). Increasing the plane of nutrition, for 4 days before and 1 day after mating, resulted in considerable improvement in fertility of rabbits (Broeck and Lampo, 1978).

Mechanisms, by which nutrients influence reproduction, are mainly through the higher neural centers or hypothalamus, via such mediators as glucose, insulin, amino acids and a number of neurotransmitters (Smith and Somade, 1994). Nutrient digestibility and utilization may therefore play an important role in the availability of these mediators during reproduction. The level of protein in the diet might have more effect on hepatic enzymes than the level of energy (Thomas *et al*., 1987). This is because a reduction in either quality or quantity of dietary protein depresses the level of microsomal mixed function oxidase enzymes in the liver (Campbell and Hayes, 1974). A significant positive correlation exists between protein content of feed and crude protein digestibility (Fekete and Gippert, 1986; Villamide and Fraga, 1998). This implies therefore, that the higher the protein content of the diet,

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the higher the digestibility of protein by rabbits. This could translate into more amino acids in circulation during flushing, which might consequently result in higher ovulation rates and litter size. No previous work done on the effect of flushing on nutrient digestibility of rabbits has been reported. This study was therefore conducted to investigate the effect of flushing using different protein levels on body weight changes, nutrient digestibility and litter size of nulliparous does.

MATERIALS AND METHODS

Study Site

The experiment was conducted in the National Animal Production Research Institute (NAPRI), Shika, Nigeria (10° 11' N, 7° 8' E and 650 m above sea level). The area receives an annual rainfall of 1100 mm, spread from April to October. Mean minimum and maximum temperatures range between 12-28°C during the cold (harmattan) season and 20-36°C in the hot season. Relative humidity during the wet season is about 75 and 21% during the dry season.

Animals

Twenty-eight nulliparous New Zealand White X California does aged 7 months were used for this study. All does were housed individually in metal cages with dimensions of 120×60×50 cm. The cages were kept in a completely walled house with open windows covered with poultry wire mesh and mosquito netting.

Experimental Procedure

The does were randomly assigned to four diets containing 18, 20, 22 or 24% CP (Table 1) in a completely randomized design. The does were routinely fed 18% CP diet prior to the study. During the study, the does were flushed for 1 week before mating and 1 week after mating. All does were fed 100 g of the concentrate diets daily during flushing at 08.00 h. After flushing, all the does were placed on the 18% CP diet for the remaining period of pregnancy and during lactation up to 4 weeks after kindling when the kits were weaned. The does were supplied 150 g of the 18% CP diet during pregnancy and 350 g during lactation. The diets were fed in meal form to the does. Water was supplied *ad libitum*. Feed and water were provided in flat-bottom earthen pots. Feed wastage and left over were monitored daily. Feed intake was determined by subtracting feed wastage and leftover from the total feed offered. The earthen feeders used drastically reduced feed wastage.

Table 1: Composition of experimental diets fed to does

	Treatments (% CP)			
Parameters	18	20	22	24
Maize	48.00	45.73	39.24	32.76
Groundnut cake	33.70	35.77	42.26	48.74
Maize offal	15.00	15.00	15.00	15.00
Bone meal	2.80	3.00	3.00	3.00
Salt	0.25	0.25	0.25	0.25
Vit/Min. premix*	0.25	0.25	0.25	0.25
Chemical composition				
Energy (kcal ME kg^{-1})**	2638.90	2687.74	2627.43	2567.19
Crude protein (%DM)	18.75	20.94	22.38	24.75
Crude fibre (%DM)	15.24	13.22	12.66	10.20
Ash (%DM)	6.80	6.63	6.00	5.50
Ether extract (%DM)	3.79	4.34	4.71	4.88
Nitrogen free extract (%DM)	55.42	54.87	54.25	54.67

*: Vitamin/mineral premix content per kilogram ration: Vit. A 1251 IU, Vit. D3 2750 IU, Vit. E 151 IU, Vit. K 0.002 g, Vit. B_2 0.006 g, Nicotinic acid 0.035, Calcium D-Pantothenate 0.01 mg, Vit. B_6 0.0035 g, Vit. B_{12} 0.02 g, Folic acid 0.001 g, Biotin 0.0005 g, Vit. C 0.025 g, Cholin chloride 0.39 g, Zinc bacitracin 0.02 g, Methionine 0.2 g, Avatec (Lasolocid) 0.09 g, Manganese 0.1 g, Iron 0.05 g, Zinc 0.04 g, Copper 0.002 g, Iodine 0.00153 g, Cobalt 0.000225 g, Selenium 0.0001 g. **: Energy was calculated

Does were mated to intact bucks in the morning. Pregnancy was determined by weight and palpation methods. Nesting earthen pots were supplied to rabbit does on the 25th day of pregnancy. Does were weighed at onset of flushing, mating, end of flushing and kindling. Litter size and weight of kits were monitored at birth and at weekly intervals up to weaning at 4 weeks after kindling. The study lasted 12 weeks.

Faecal samples were collected for 4 days in the second week of flushing from six does per treatment. Samples collected were stored at -20°C in a deep freezer immediately after collection. The samples were pooled together for each rabbit and analyzed for proximate composition.

Analytical Methods

Dry matter content of the diets and faeces was determined after pre-drying the samples at 60°C for 24 h and then at 105°C in a vacuum oven. Ether extract was determined using Soxhlet extraction procedure. Crude fibre was determined as the fraction remaining after digestion with standard solutions of Sulphuric acid and Sodium hydroxide under controlled conditions. Ash (minerals) was determined by ashing at 550°C. Total nitrogen contents of the diets and faeces were determined by the macro-Kjeldahl method. The nitrogen content of the diets and faeces was converted to crude protein by multiplying 6.25. Proximate analysis was carried out according to AOAC (1980).

Data Analysis

Apparent Digestibility Coefficients (ADC), Digestible nutrients (Dn) intake and Total Digestible Nutrients (TDN) were calculated as:

$$\text{ADC (\%)} = \frac{\text{Nutrient intake} - \text{Nutrient in feaces}}{\text{Nutrient intake}} \times 100$$

$$\text{Dn (g kg}^{-1}\text{)} = \text{ADC (\%)} \times \text{Nutrient in feed}$$

$$\text{TDN (\%)} = \text{Dnx}_1 + \text{Dnx}_2 + \text{Dnx}_3 + (\text{Dnx}_4 \times 2.25)$$

Where:

x_1 = Crude protein (%),
x_2 = Crude fibre (%),
x_3 = Nitrogen free extract (%),
x_4 = Ether extract (%).

Total milk production of the does was calculated according to Parigi Bini and Xiccato (1998) as:

$$\text{TMP (kg)} = 1.77 + 1.39\ \text{LW21}$$

Where:

TMP = Total Milk Production,
LW21 = Litter Weight at 21 days (kg).

Data collected were subjected to analysis of variance test using PROC GLM and significant differences between means were separated using pair-wise difference (pdiff) method (SAS, 1987). Polynomial (trend) analysis was carried out to determine the trend of response to level of protein for flushing using the model;

$$Y = a + bx + cx^2$$

Where:
Y = Trait of interest,
x = Protein level.

The minimum level of protein was determined using; $\frac{-b}{2c}$

RESULTS AND DISCUSSION

Body weight of does at the end of flushing and kindling were not affected by protein level (Table 2). Similar non-differences in doe weights at kindling have been reported by Sanchez *et al.* (1985) and Smith *et al.* (1990) in ewes. Reharjo *et al.* (1986) however, reported significant differences in mean doe weights at kindling when fed 16 and 21% CP. Doe weight gain during flushing was not significantly different for all the treatments. In the first week of flushing (weight gain at mating), weight gain was minimal for all the treatments except 20% CP. This low weight gain could be as a result of adjustment of the rabbit does to the various protein levels. At the end of flushing (second week of flushing) however, weight gain increased with increase in protein level. Similar non-significant differences in mean daily weight gain of does fed different sources and levels of protein during pregnancy have been reported by Aganga *et al.* (1991), while Jindal *et al.* (1996) reported similar mean weight gain of sows during the first 15 days of pregnancy despite differences in feed intake imposed. Observed increase in doe weight gain with increase in protein level in this study could be expected, as high protein intake would make available more amino acids for utilization by the animal.

Doe feed intake (Table 2) was higher before mating than after mating (at the end of flushing) and showed a decreasing trend with increase in protein level up to 22%. There was a reverse in the trend after mating with the does eating less feed than before mating. Pregnancy could have resulted in a lowering of the doe's appetite. Doe feed conversion efficiency was higher after than before mating indicating a likely improvement in efficiency of feed utilization of does during pregnancy.

Ash digestibility increased with increase in protein level (Table 3). There was negative crude fibre digestibility with increasing protein level except for 20% CP indicating non-digestion of this nutrient. Fekete and Gippert (1986) reported similar non-digestion of crude fibre in their study. Rabbits are poor digesters of crude fibre resulting from selective retention of small particles (protein) rather than large particles (fibre) in the ceacum which are excreted in the hard faeces (Cheeke *et al.*, 1986). Does flushed with 24% CP digested 25-71% more crude protein compared with the other treatments. Significant ($p<0.001$) differences in nitrogen digestibility were reported for does fed 13.5, 17.5 and 21.0% protein diets (Partridge and Allan, 1982). Their values of 0.641, 0.691 and 0.695, respectively, are similar to those obtained in this study.

Table 2: Effect of flushing on performance of nulliparous does

	Crude protein (%)				
Parameters	18	20	22	24	SEM
Initial weight (kg)	2.43	2.27	2.14	2.42	0.35
Weight after flushing (kg)	2.49	2.35	2.36	2.56	0.38
Weight at kindling (kg)	2.41	2.22	2.31	2.55	0.37
Weight change at mating (g)	-16.67	125.00	12.50	50.00	43.59
Weight change at end of flushing (g)	91.67	133.33	162.50	182.00	33.87
Feed intake at mating (g)	628.17	603.33	556.25	568.00	38.81
Feed intake at end of flushing (g)	475.00	526.67	533.75	482.00	31.10
Feed gain at mating	-4.91	7.95	7.82	11.62	7.23
Feed gain at end of flushing	2.56	3.14	3.81	4.18	0.91

Table 3: Apparent digestibility coefficients (%) of nutrients of nulliparous does flushed with different levels of protein

	Crude protein (%)				
Parameters	18	20	22	24	SEM
Ash	12.32	13.47	31.34	38.45	10.6
Dry matter	55.77	45.83	53.33	58.36	6.3
Ether extract*	44.09[a]	24.68[b]	44.44[a]	55.14[a]	8.5
Crude fibre	-5.83	17.18	-1.83	-13.26	11.8
Crude protein**	64.01[bc]	49.57[c]	67.65[b]	85.00[a]	7.4
Nitrogen free extract*	76.92[a]	59.61[b]	62.37[b]	58.39[b]	5.1
Total digestible nutrients	56.97	45.84	53.66	58.99	6.8

Means with different superscript on the same row are significantly different, *: $p<0.05$, **: $p<0.01$, SEM: Standard Error of Mean

Table 4: Digestible nutrients (g kg^{-1}) intake of nulliparous does flushed with different levels of protein

	Crude protein (%)				
Nutrients	18	20	22	24	SEM
Crude protein**	145.6[bc]	123.7[c]	178.5[b]	252.8[a]	13.61
Crude fibre	-8.9	31.3	-2.3	-13.5	18.88
Ether extract*	16.6[bc]	10.7[c]	20.9[ab]	26.9[a]	3.63
Nitrogen free extract*	395.5[a]	279.4[b]	313.4[b]	290.0[b]	21.91

Means with different superscript on the same row are significantly different, *: $p<0.05$, **: $p<0.001$, SEM: Standard Error of Mean

Table 5: Effect of flushing on litter size and total milk production of nulliparous does

	Crude protein (%)				
Parameters	18	20	22	24	SEM
Litter size					
At birth	6.00	4.83	4.40	7.75	1.67
Alive at birth	5.00	4.50	4.00	7.25	1.78
Day 7 postpartum	4.67[a]	4.44[a]	0.40[b]	7.25[a]	1.67
Day 21 postpartum	4.50[a]	4.00[a]	0.40[b]	7.25[a]	1.71
Litter weight					
Litter birth weight (g)	275.00	218.33	165.00	250.12	72.30
Litter weight alive at birth (g)	258.33	201.67	155.00	250.04	64.98
Kit birth weight (g)	45.19	45.19	30.71	34.49	7.80
Day 21 postpartum (g)	925.00[a]	991.70[a]	170.00[b]	940.00[a]	360.71
Total milk production (kg)	3.06[a]	3.15[a]	2.01[b]	3.08[a]	0.50

Means with different superscript on the same row are significantly different ($p<0.05$), SEM: Standard Error of Mean

Does flushed with 24% CP had significantly higher digestible crude protein and ether extract intake than the other treatments (Table 4). This could indicate higher availability of amino acids for protein accretion and lipids for fat accretion in does flushed with 24% CP. Results from this study showed a slightly though non-significantly higher weight gain of does flushed with 24% CP diet. Does flushed with 18% CP digested significantly ($p<0.05$) more nitrogen free extract than those in other groups.

Flushing had non-significant effect on litter size (Table 5) at birth ($p = 0.07$) and alive at birth ($p = 0.06$). This indicates that though there was an increase in digestibility of nutrients with increase in protein level during flushing, this has not translated into significant increase in ovulation rate. Iyeghe-Erakpotobor *et al*. (2002) reported similar non-significant effect of flushing on litter size of multiparous does. Partridge (1989) reported very minor or no effect of variations in nutrient intake on ovulation rate in rabbits. Altering dietary protein levels in pigs also, did not enhance ovulation rates above the conventional diets (Robinson, 1990). Since amino acids impinge on reproduction through the higher neural centers (Smith and Somade, 1994), it is expected that the higher protein digestibility and intake observed in this study should have elicited higher litter sizes especially on the higher protein levels (22 and 24% CP), by increasing the level of mixed function oxidases and increasing ovulation rate (Thomas *et al*., 1987). This does not appear to be the case in this study.

Results from polynomial analysis for litter size at birth (LSB) and litter size alive at birth (LSA) indicated a significant quadratic trend supported by the following equations:

$$LSB = 123.75\ (\pm 44.69) - 11.61\ (\pm 4.30)\ x + 0.28\ (\pm 0.103)\ x^2 \qquad p = 0.03$$

$$LSA = 113.11\ (\pm 46.87) - 10.75\ (\pm 4.51)\ x + 0.26\ (\pm 0.108)\ x^2 \qquad p = 0.04$$

Where:

x = Level of protein fed to does (% CP).

Standard error in parenthesis.

From the study, level of protein with the lowest litter size at birth and alive at birth are 20.58 and 20.34% CP, respectively.

The fact that does flushed with 24% CP diet had lower rearing losses might indicate higher milk production by these does compared with the other treatments. This was not the case as total milk production was similar for does flushed with 18, 20 and 24% CP. This could be because the period of higher protein accretion (as evidenced by the weight gain of does) during flushing was too short to elicit a measurable buildup of body tissue reserves for milk production. A relationship between rate of tissue catabolism for milk production and level of tissue reserves available for catabolism at parturition has been established (Hughes, 1993).

Litter size at day 7 and 21 was significantly ($p = 0.02$) higher for does flushed with 24, 20 and 18% CP than 22% CP. Very high mortality of kits observed for does flushed with 22% CP in the first week after kindling could be due to low kit birth weight and/or inexperience as the does were rearing kits for the first time. An earlier study with multiparous does did not record such mortality with 22% CP (Iyeghe-Erakpotobor ***et al***., 2002). It is concluded, that flushing rabbits with protein did not significantly affect body weight changes, feed intake and feed conversion efficiency or litter size at birth. Though significant positive digestibility of crude protein, nitrogen free extract and ether extract were observed, the duration of flushing was too short to result in an appreciable build up of doe body reserves to elicit significant change in litter size.

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