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Nutritional and haemato-biochemical modulation in dairy goats during mid-pregnancy

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ABSTRACT

The present study was conducted to assess the influence of mid-pregnancy on the nutrient utilization, haematological and blood biochemical profile in gravid goats. Sixteen indigenous non-descript does of approximately 3–4 years of age were randomly allotted to two equal groups (PREG and NPREG) of eight each based on individual BW. The goats of pregnant group (PREG) were synchronized, mated and were left for routine group feeding, care and management. They were brought to experiment just 60 days after mating along with the non-pregnant (NPREG) group; both the groups were offered basal diet comprised of concentrate mixture and wheat straw to meet their nutrient requirements. A metabolic trial of six day duration was conducted at 90 days after mating on all the experimental goats to assess the nutritional modulation along with any changes of haematological and blood biochemical parameters at mid pregnancy stages. The results revealed no variation in intake and digestibility of DM, OM and CP. However, the digestibility of NDF, ADF and hemicellulose was higher for PREG does as compared to NPREG control. The balance of nitrogen was positive and comparable between the groups. Blood haemato-biochemical profile showed no significant influence of pregnancy on different blood metabolites except that of low serum urea. The serum variables representative of the liver function was within the normal range and were comparable between the groups. It is concluded that mid-pregnancy induces no perceptible nutritional modulation except for subtle improvements in fibre digestibility, and possibly protein utilization in dairy goats.

Key words: Blood metabolites, Goat, Haematology, Mid-pregnancy, Nutritional modulation

The nutritional requirement during pregnancy of mammals currently is an intensive area of investigation and the pool of information is growing fast. There has been much interest over the past decade regarding pregnancy nutrition and its impact on animal health, reproduction, lactation performance and also the health of new-born. Requirements for pregnancy represent nutrient amounts necessary to support both growth rate and maintenance of foetus and placenta. Additionally, there is development of maternal tissues (e.g., uterus and mammary gland) for nutritional support of the conceptus pre- and post-natally, respectively. Inappropriate maternal and foetal nutrition at different developmental stages may likely have specific short- and long-term detrimental effects (Godfrey and Robinson 1998). Many studies have been carried out earlier at different stages of pregnancy, viz. early, mid and late pregnancy period in various livestock species which confirm the need of judicious use of nutrient at different stages for making the livestock production profitable. Accurate feeding and good management systems during pregnancy period are important both for mother and growing foetus. However, there is scarce information in caprine regarding nutritional impact on pregnancy and other productive parameters. Early stage of pregnancy require little or no extra nutrient as maintenance diets are enough to fulfill the requirement of both mother and slow growing foetus. It is a well-established fact that late gestation period requires special attention on nutritional aspect as foetus develops rapidly during this period to acquire up to 75 to 80% of their future birth body weight (Fthenakis *et al.* 2012). However, there is little research on the nutritional aspects during mid pregnancy.The need of increased nutrition above the maintenance level during mid gestation is controversial. Most of the researchers confirm no extra requirement above the maintenance level during this period. The two most prominent publications on the nutrient requirements of goats (NRC 2007, Kearl 1982) have given no special consideration for the nutritional need of mother and foetus in early and mid gestation for goats. In fact, NRC (2007) has defined the requirements during gestation period starting from day 105, and Kearl (1982) has suggested the same only during the last eight weeks of gestation. However, Terrazas *et al.* (2012) and Blache *et al.* (2007) reported slight

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increase in nutrient requirements of dams during early and mid-pregnancy than those at maintenance to provide a suitable uterine environment for the early development of the embryo and foetus. A number of studies have shown that restricted nutrition of ewes in early- to mid-pregnancy can inhibit optimal placental growth (McGrab *et al.* 1992, Clarke *et al.* 1998) which will unable to nourish the foetus adequately in final stage of pregnancy causing reduced birth weight. Furthermore, as foetal growth is more due to hypertrophy than to hyperplasia with increase in foetal age, it has been suggested that retardation of foetal growth late in gestation should have less severe effect on subsequent neonatal development than retardation at an early stage (Prior and Laster 1979). So, the care of animals at initial stages (both early and mid pregnancy stages) is equally important as that of late stage of pregnancy close to parturition. There is need for accurate expressions of the nutrient requirements in mid gestation. More feeding trial data are required to be generated on nutrient utilization and requirement in mid gestation to establish the dilemma of controversy. Thus, the present investigation was undertaken with the objective to investigate the way the mid pregnancy influences the nutrient utilization, haematological and blood metabolic profile in gravid goats.

MATERIALS AND METHODS

Animals, experimental design and diets: The experiment was conducted at Animal Nutrition Research Sheds of Indian Veterinary Research Institute (IVRI), Izatnagar, Uttar Pradesh, India. Proper ethical care and management procedures were adopted throughout the study period according to the guidelines of institutional animal ethics committee for experimentation constituted as per the article number 13 of the CPCSEA rules, laid down by the Government of India.

Sixteen indigenous non-descript does, approximately 3–4 years of age, were randomly allotted to two equal groups (PREG, NPREG) of eight each based on their individual body weight. Randomly selected female goats of pregnant group (PREG) were synchronized with $PGF_{2\alpha}$ at a total dose of 7.5 mg (Lutalyse® @ 1.5 ml/goat; repeated after 11 h) and were mated to selected sire for making them pregnant and left for routine group feeding, care and management as followed in institutional sheep and goat farm. Another group (NPREG) of female non-pregnant animals served as control. Animals of both the groups were brought to experiment just 60 days after mating and housed in a well ventilated concrete-floored shed with facilities for individual feeding and watering. Prior to this, the animals were fed a standard basal diet for a period of 10 days so as to adapt them to the new feeding and management conditions. The offered basal diet comprised concentrate mixture and wheat straw to meet their nutrient requirements as per Kearl (1982). The concentrate mixture consisted of maize (30%), soybean meal (30%), wheat bran (37%), mineral mixture (2%) and common salt (1%). Weighed quantity of concentrate mixture was provided daily once a day in early morning to meet almost their whole CP and major part of the energy (ME) requirement. Wheat straw offered *ad lib.* shortly along with concentrate mixture to meet the appetite of the animals. Water was provided thrice a day in morning, afternoon and evening in bucket filled with water.

Metabolism trial: A metabolism trial of 6 days was conducted on all the experimental goats following 30 days of experimental feeding (at about 90 days into pregnancy) to study the digestibility, plane of nutrition and nitrogen retention. The trial involved daily recording of feeds offered and residues left besides total excretion of faeces and urine after allowing for proper acclimatization of goats in metabolic cages. Representative samples of feeds offered and residues left were dried overnight at 100±5°C in hot air oven to determine daily dry matter (DM) intake. The samples were pooled over the 6 days of collection and then ground to pass through 2 mm sieve, and stored in airtight containers for further analysis. The total quantity of faeces voided by individual goats during the preceding 24 h was collected and weighed quantitatively. Following thorough and uniform mixing in a clean plastic trough, representative samples were taken to the laboratory for further aliquoting. A suitable aliquot of the daily faecal excretion was then dried in hot air oven at 100±2°C for determining the DM content. The dried samples of faeces of individual goats was subsequently pooled, ground and secured for further analysis as in case of feeds. For nitrogen (N) estimation, an appropriate aliquot of the wet (fresh) faeces was weighed, mixed with few ml of 1:4 H_2SO_4 and pooled into a previously weighed air tight container. At the end of the collection period, the pooled aliquot of the wet faeces was weighed and mixed well, and used for N estimation. The daily urinary output by individual goats was collected quantitatively in plastic bottles and measured. Suitable aliquots, in duplicate, were measured daily into kjeldahl flasks containing concentrated H_2SO_4 for N estimation. The proximate analysis of feed, faeces, residues, and N content of urine was performed as per AOAC (1990).

Blood sampling and analysis: Blood samples were collected from jugular vein from all animals after the metabolism trial into two tubes—one with, and the other without anticoagulant. They were brought to the laboratory in chilled ice boxes soon after collection. The anticoagulated blood was used for haematologic analysis using an automated haematology cell counter (MS4s, Melet Schloesing Laboratories, France). The non-anticoagulated blood samples were centrifuged at 3,000×g at 4°C for 20 min to separate the serum. The collected serum samples were stored at –20°C until further analysis for estimating glucose by O-toluidine method (Hultman 1959), total protein (Biuret method), albumin (bromocresol green method) and globulin (by difference) as per Webster (1977), urea by diacetyl monoxime (DAM) method (Wybenga *et al.* 1971), uric acid (Phosphotungstate method) and total cholesterol (Wybenga *et al.* 1970).

Statistical analysis: The data generated during the study

Table 1. Chemical composition of experimental feeds on %DM basis

Attribute	Concentrate mixture†	Wheat straw
Dry matter	91.62	91.43
Organic matter	93.59	95.46
Crude protein	23.63	3.71
Ether extract	2.00	2.71
Total carbohydrates	67.96	89.04
Total ash	6.41	4.54
Neutral detergent fibre	30.07	85.78
Acid detergent fibre	11.19	56.27
Hemicellulose	18.88	29.51

†Contained (per 100 kg): maize 30, soyabean meal 30, wheat bran 37, mineral mixture 2 and common salt 1 kg.

Table 2. Mean daily intake and digestibility of nutrient in treatment groups

Attribute	Dietary groups†		P value
	PREG	NPREG	
Feed (DM) intake			
Concentrate (g)	274.87±0.00	274.87±0.00	1.00
Wheat straw (g)	242.99±16.99	210.13±17.53	0.20
Total, g	517.86±16.99	485.00±17.53	0.20
g/kg BW	26.03±0.74	26.40±1.08	0.78
g/kg $W^{0.75}$	54.92±1.31	54.54±1.73	0.86
Concentrate: roughage ratio	1.18±0.09	1.37±0.12	0.19
Digestibility (%)			
Dry matter	62.16±1.01	62.01±0.65	0.90
Organic matter	64.65±1.09	64.39±0.59	0.84
Crude protein	72.28±1.37	72.98±1.95	0.77
Ether extract	84.98±1.50	81.59±0.87	0.07
Total carbohydrates	62.63±1.27	62.08±0.70	0.71
NDF	50.19^{a}±1.03	44.05^{b}±1.70	0.01
ADF	45.00^{a}±0.92	38.26^{b}±2.14	0.01
Hemicellulose	57.41^{a}±1.60	51.45^{b}±1.81	0.03
Nutrient intake			
DDM (g)	320.88±6.72	300.28±9.16	0.09
(g/kg $W^{0.75}$)	34.08±0.69	33.82±1.10	0.84
DOM (g)	315.08±6.49	293.27±9.63	0.08
(g/kg $W^{0.75}$)	33.46±0.62	33.01±1.07	0.72
CP (g)	75.70±0.39	74.90±0.41	0.18
(g/kg $W^{0.75}$)	8.06±0.23	8.47±0.31	0.31
DCP (g)	54.69±0.94	54.63±1.35	0.97
(g/kg $W^{0.75}$)	5.83±0.22	6.19±0.29	0.34
ME (kcal)	1130±23.2	1051±34.5	0.08
(kcal/kg $W^{0.75}$)	119.96±2.24	118.34±3.83	0.72
Nutritive value of composite diet			
CP (%)	14.71±0.41	15.56±0.47	0.20
DCP (%)	10.66±0.43	11.39±0.55	0.31
DOMD (%)	61.04±1.01	60.53±0.53	0.66
ME (Mcal/kg)	2.19±0.04	2.17±0.02	0.65

†PREG, Pregnant; NPREG, Non-pregnant control group. [ab]Means with different superscripts in a row differ significantly (P<0.05).

were analyzed using one-way analysis of variance (ANOVA). The means were subjected to test of significance by Duncan's multiple range test as described by Snedecor and Cochran (1989) using IBM Statistical Package for Social Science (IBM SPSS 20.0) software. The data were expressed as mean±SE, and all statements of significance are based on a probability of P<0.05 unless otherwise indicated.

RESULTS AND DISCUSSION

Chemical composition of feeds: The chemical composition of concentrate mixture and wheat straw used in this experiment is shown in Table 1. The chemical composition of basal feed of concentrate mixture and wheat straw was closer to the values reported earlier in pregnant goats (Rastogi *et al.* 2003, Patra 2001).

Intake and digestibility of nutrients: The data on intake and digestibility of various nutrients are presented in Table 2. The mean daily intake of concentrate mixture was similar (P>0.05) across the groups as each of the animal was offered an equal weighed amount of the concentrate mixture, which was consumed completely by the animals. The wheat straw was offered *ad lib.* and the observed variation in total DM intake was completely due to differences in the intake in wheat straw. However, no significant (P>0.05) variation was observed among the group in wheat straw intake and total DM intake both in term of absolute value and g/kg body size. The DM intake was maintained almost similar among both group suggesting no reduction in volume of the abdominal cavity in pregnant group, which limits potential distention of the digestive tract and thus the amount of feed intake.

There were no variations in the digestibility of DM, OM, CP, EE, TCHO apparent between the two groups. However, the digestibility of NDF, ADF and hemicellulose was higher (P<0.05) for pregnant does as compared to the non-pregnant does. This could be attributed to higher wheat straw consumption compared to control, as could be ascertained from the lower concentrate:roughage (C:R) ratio of pregnant animal. Moreover, lower C:R ratio lead to slower rate of passage of digesta causing more retention time in intestine for better action by microbes for its digestion. These results were in contrast to the reports indicating that higher C:R ratio may have resulted in better digestibility of DM and OM (Singh and Singh 1990, Kawas *et al.* 1991, Patra 2001).

The daily intake of various nutrients did not differ significantly between the pregnant and non-pregnant control group. There is no increase of energy demand by conceptus. Intake of DDM and DOM were comparable among the groups. However, there was an improving trend observed for both DDM (P=0.09) and DOM (P=0.08) intake for animals in the pregnant group. A similar increasing trend (P=0.08) in ME intake was also observed when gravid does at mid pregnancy stages was compared against non-pregnant control group. This may be the reflection of a slight increase in higher demand of nutrient by pregnant animals

Table 3. Nitrogen metabolism in goats of different treatment groups

Attribute	Dietary groups†		P value
	PREG	NPREG	
Nitrogen intake (NI) (g)	12.11±0.06	12.23±0.04	0.13
(g/kg $W^{0.75}$)	1.29±0.04	1.39±0.05	0.17
Nitrogen excretion			
Faecal, g	3.36±0.18	3.24±0.24	0.70
% NI	27.72±1.37	26.49±1.94	0.61
Urinary, g	6.57±0.21	6.35±0.21	0.47
% NI	54.24±1.62	51.94±1.69	0.34
Nitrogen retention			
Balance	2.18±0.26	2.63±0.32	0.29
(gg/kg $W^{0.75}$)	0.23±0.03	0.30±0.04	0.22
% NI	18.04±2.15	21.57±2.68	0.32
% NA	24.76±2.69	28.93±3.16	0.33

†PREG: Pregnant, NPREG: Non-pregnant control group.

Table 4. Haematological parameters in goats under different treatment groups

Attribute	Dietary groups†		P value
	PREG	NPREG	
TLC (10^3/mm^3)	7.44±0.68	6.64±0.75	0.44
Lymphocyte (%)	60.21±0.83	59.78±0.86	0.72
(10^3/mm^3)	4.46±0.38	3.96±0.46	0.42
Monocyte (%)	4.69±0.63	5.69±0.75	0.33
(10^3/mm^3)	0.34±0.06	0.36±0.05	0.77
Neutrophil (%)	35.10±0.54	34.54±0.43	0.43
(10^3/mm^3)	2.63±0.28	2.33±0.29	0.47
RBC (10^6/mm^3)	10.16±0.25	10.01±0.35	0.73
Haematocrit (%)	25.51±0.85	25.76±1.09	0.86
Haemoglobin (g/dl)	9.69±0.34	9.61±0.51	0.90
Thrombocytes (10^3/mm^3)	398.5±48.0	373.8±33.2	0.68

†PREG: Pregnant, NPREG: Non-pregnant control group.

for the foetus and the developing conceptus. But this increased demand is not very high and could be effectively taken care by the animals by an observed 15% increase in the voluntary consumption of feed (wheat straw). The intake of CP and DCP were comparable between the two groups; protein which is the most important nutrient for growth and tissue deposition also remains similar between the pregnant and non-pregnant groups. Incidentally, goats under both the groups had DCP intake well above the recommended maintenance requirement of 2.51 g/kg $W^{0.75}$ (Kearl 1982). The nutritive value of composite diets in terms of per cent content of DCP, DOMD and ME in both the group was comparable.

Nitrogen metabolism: Data pertaining to nitrogen metabolism is presented in Table 3. The mean daily intake of nitrogen was 12.11±0.06 and 12.23±0.04 in pregnant and non pregnant control group, respectively, without any significant variations (P>0.05). The faecal and urinary excretion of nitrogen was almost similar among the groups. The major part of nitrogen (≥50% of NI) is excreted through urine which is almost double of that of faecal excretion (~ 25% of NI). This resulted in only 18–22% of the ingested nitrogen being retained by the does, irrespective of pregnancy. The comparable higher loss through urinary routes resulted in only 25–29% of the absorbed nitrogen being bio-available to the animals. It could possibly be due to comparatively higher protein consumption in relation to energy. In fact, does in the control and pregnant group consumed about 52 and 48 g DCP per Mcal ME, which were much higher than the suggested maintenance requirement of ~28 g DCP/Mcal ME (Kearl 1982). All the does were in positive nitrogen balance, indicating adequate nutritional level of both groups. Nitrogen balance, expressed either in absolute terms or in relation to metabolic body size, showed a similar (P<0.05) retention in both groups, expressing no impact of pregnancy during present stage. There is apparently no extra demand by the foetus for its tissue growth during this stage (mid-pregnancy) beyond the maintenance diet.

Haematological changes: Haematological parameters (Table 4) are helpful to determine the health, nutritional status and general well being of animals (Gupta *et al.* 2007, Radostits *et al.* 1994). The levels of RBC, Hb and haematocrit were within the normal ranges as reported by Keneko *et al.* (1997) and were comparable between the two groups. Similar observation was also reported by Roy *et al.* (1965) who found no deviation of Hb and haematocrit from normal value up to 120 days of gestation period in goats. It had been reported that Hb and haematocrit levels gradually fall as the animal approaches parturition due to developing foetus and mobilization of maternal Hb into the foetal circulation by the breakdown of maternal red blood cells and subsequent transfer of pigments (Prakash and Tandon 1978, Purohit *et al.* 2000). No such observation was apparent in the present experiment.

The total leukocyte counts observed in the present study were close to other reports in goats by other researchers (Vrzgula *et al.* 1985). The TLC between the group was comparable and had no significant (P>0.05) difference, which was in contrast to the previous report of Fortagne and Schafer (1989) and Sandabe and Yahi (2000), who reported an increase in the TLC of pregnant goats. This may be attributable to variations in ages, sample size, environmental changes and different stage of pregnancy. There was also no differences (P>0.05) between the groups in the differential leukocyte count and thrombocytes.

Blood biochemical profile: The blood biochemical profile of goats measured during the experiment is presented in Table 5. All the values obtained for the measured variables were well within the normal range reported for goats (Kaneko *et al.* 1997, Radostits *et al.* 2007), which is a reflection of nutritional adequacy and completeness. There was no difference between the two groups with regard to the parameters (except for serum urea), indicating the absence of any perceptible impact of mid-pregnancy on the metabolic status of the animals. Davis and Johnston (1971)

Table 5. Blood biochemical parameters in goats of different treatment groups (PREG- Pregnant and NPREG- Non-pregnant control group)

Attribute	Dietary groups†		P value
	PREG	NPREG	
Glucose (mg/dl)	43.23±4.16	43.81±2.38	0.90
Total protein (g/dl)	7.78±0.29	6.98±0.52	0.20
Albumin (g/dl)	3.26±0.11	3.10±0.17	0.47
Globulin (g/dl)	4.53±0.34	3.88±0.47	0.28
A:G ratio	0.76±0.08	0.90±0.15	0.43
Uric acid (mg/dl)	0.78±0.11	0.72±0.10	0.68
ALT (IU/l)	26.85±2.14	35.21±5.98	0.21
AST (IU/l)	87.10±7.03	74.29±10.44	0.33
Urea (mg/dl)	27.61^{b}±1.14	34.29^{a}±1.00	<0.01
Total cholesterol (mg/dl)	140.56±6.22	128.31±5.71	0.17
HDL cholesterol (mg/dl)	35.14±1.56	32.08±1.43	0.17

†PREG: Pregnant, NPREG: Non-pregnant control group. [abc]Means with different superscripts in a row differ significantly.

reported that foetal requirement for energy is supplied primarily by maternal glucose. But no significant decrease of glucose level was observed in the pregnant animals in comparison to control. However, there was significant (P>0.05) increase in urea concentration observed in serum of non pregnant group which may be attributed to increase supply of CP level of diet than that of its requirement, especially in relation to energy (ME), as discussed under the nitrogen metabolism section. Furthermore, the similar level of protein intake with low urea excretion in pregnant group suggest high rate and better utilization of protein by the developing foetus from amino acid derived from its mother.

The serum variables representative of the liver function, viz. total protein, albumin, globulin, A:G ratio, ALT and AST did not exhibit any variations attributable to pregnancy. The plasma protein is sensitive to nutritional influences but the changes are often subtle and difficult to detect and interpret. The level of serum proteins depends upon several factors including extent, duration and nature of the hepatic disorder, the inflammatory or metabolic hepatic processes and the presence of other organ disorders (Kaneko *et al.* 1997). It has been reported that albumin decrease to a minimum at mid gestation (Dunlap and Dickson 1955). But no such observation was recorded in present experiment. Moreover, A:G ratio remain within the normal range (Kaneko *et al.* 1997) irrespective of mid pregnancy stage.

The present study indicated no differences in the nutrient metabolism suggestive of possible higher requirements and/ or bio-availability during mid-pregnancy in goats and can be effectively taken care by the stipulated maintenance allowance. One of the reasons for such a finding could be the confounding influence of higher dietary protein in the present experiment. However, further studies using graded levels of major nutrients like energy and protein could be a better approach to highlight the subtle differences, if any, in their requirements in early stages of pregnancy.

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