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Effect of concentrates and probiotic supplementation on blood biochemical indices of boar x local cross periparturient goats

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Abstract
Present study was conducted at MRCSG Shuhama to study the effect of concentrate and probiotics supplementation on the performance of does during periparturient period. Twenty four pregnant healthy Boar x local cross does in the last month of gestation (day 120) were selected randomly and allotted to 4 treatment groups (T1, T2, T3 and T4) of 6 animals each. The does were maintained under stall feeding conditions and offered a daily ration consisting of oats hay @ 1.2 kg/head/day and commercial pelleted feed @ 577.5 g/head/day during periparturient period (one month pre-partum to one month post-partum). Does in treatment groups T1 were offered normal daily ration without supplementation. In T2 group were offered normal ration with concentrate @ 150 gram /head/day, T3 group were offered normal ratio with concentrate @ 150 gram/head/day and probiotic @ 2.5 gram/head/day and in T4 groups were offered normal ratio with concentrate 150g + probiotic @ 4 gram/head/day. Probiotic = *Saccharomyces cerevisiae* x10^{10} CFU. There was a significantly (p<0.05) higher plasma glucose (mg/dl) and protein (g/dl) concentration in T2, T3 and T4 as compared to T1 (control) treatment group at kidding and after kidding however there was non-significant difference between T2, T3 and T4 treatment group. At end of trail highest plasma glucose concentration was observed in group T3 as 58.50±1.14 mg/dl and lowest in T1(control) as 52.00±2.64 mg/dl. At the end of trail highest plasma protein was observed in T4 as 6.11±0.38 g/dl and lowest in T1 group as 4.00±0.28 g/dl. Also, there was a non-significant difference in fortnightly plasma triglyceride and cholesterol among the different treatment groups during before kidding, at kidding and after kidding.

Keywords: Concentrates, probiotics supplementation, does, periparturient period, hematology

Introduction
Economy of Jammu and Kashmir isagriculture dependent and livestock farming occupies an important component of it. Owing to the presences of abundant alpine pasture and high demand of livestock products, sheep and goat rearing is the main activity of rural and backwardpeoples of Jammu and Kashmir union territory. It also plays a vital role in socio-economics upliftment of weaker sections of the society viz; Gujjars, Bakerwals, Chopans, Gaddies and Changpas. Goats are raised principally for their meat, milk, fibre and skin. It is very well suited with other livestock production such as sheep and cattle on low-quality grazing land (Bruinsma, 2003) [2]. Goats efficiently convert low quality grazing matter that is less desirable for other livestock into quality lean meat.

During periparturient period (three weeks before and three weeks after kidding) there is a negative energy balance in does, which is considered as primary cause for the development of the ketosis/ hyperketonemia in does resulting in their decreased performance or even mortality (Van Saun, 2000) [15]. Further during late gestation, there is reduction in the rumen capacity especially in twin and triplet-bearing animals owing to the presence of foetus and the subsequent pressure of the gravid uterus on rumen resulting in decreased dry matter intake and hence, loss of performance (Andrews *et al*., 1996) [1]. Nutrient restrictions during this period also results in foetal losses. This necessitates increasing nutrition density for meeting the requirements. Feeding of high level of concentrate increases the energy status of does during gestation and the kid born form these does are having higher body weight. Increase in the nutrient density by increasing the concentrate ratio may lead to a major change in rumen microbial populations due to rapid growth of lactic acid producing bacteria. High concentrations of lactic acid accumulation cause rumen pH to drop to less than 5.0. Probiotics supplementation during periparturient periods result in stabilization of ruminal pH, increases

fibre degradation and volatile fatty acids production and improve gut health (El-Tawab *et al.*, 2016). As such the present study was conceived to evaluate effects of concentrates and probiotics supplementation on performance of Boar x local cross does during perparturient period. Yeast mixed with concentrate stimulated the growth rate and muscle development inlambs. Yeast supplementation had a significant effect on blood haematological indices (WBC, RBC) and contributed to higher lymphocyte percentages in the leukogram, indicating that the preparation actively stimulated the immune system of lambs. (Milewski and Sobiech, 2009) [7].

Materials and Methods

The present study was conducted to explore the possibility of improvement in performance of periparturient does through concentrate and probiotics supplementation. The experiment was conducted at Mountain Research Centre for Sheep and Goat (MRCSG), Shuhama, SKUAST-K. Twenty four pregnant healthy Boar x local cross does in the last month of gestation (120 day) were selected randomly and allotted to 4 groups (T1, T2, T3 and T4) of 6 animals each. The does were maintained under stall feeding conditions and offered a daily normal ration consisting of oats hay @ 1.2 kg/head/day and commercial pelleted feed @ 577.5 g/head/day during periparturient period (one month pre-partum to one month post-partum). Does in treatment groups T1 were offered daily normal ration without supplementation. In T2 group were offered normal ration with extra concentrate @ 150 gram /head/day, T3 group were offered normal ratio with extra concentrate @ 150 gram/head/day + probiotic @ 2.5 gram/head/day and in T4 groups were offered normal ratio with extra concentrate 150g + probiotic @ 4 gram/head/day. Probiotic used in the experiment was *Saccharomyces cerevisiae* x10^{10} CFU.

Table 1: Feeding schedule of experimental animals during the study period

Different treatment	Normal ration		Extra conc (g)	Probiotics (g)	DCP (g/head/day)	TDN (g/head/day)
	Roughage oats hay(kg)	Conc(g)				
T1	1.2	577.5	-	-	105	918.6
T2	1.2	577.5	150	-	126	1015.8
T3	1.2	577.5	150	2.5	126	1015.8
T4	1.2	577.5	150	4	126	1015.8

Plasma glucose (mg/dl)

Plasma glucose was estimated by GOD-POD method (Rifai, *et al.*, 1999). The details are given below:

Principle: Glucose gets oxidized to gluconic acid and hydrogen peroxide in presence of enzyme glucose oxidase (GOD). Hydrogen peroxide (H_2O_2) so produced reacts with 4-aminoantipyrine and phenol in presence of peroxidase enzyme (POD) to produce quinoneimine which is then quantified calorimetrically.

$$\text{Glucose} + O_2 \xrightarrow{\text{GOD}} \text{Gluconic acid} + H_2O_2$$

$$2H_2O_2 + \text{4-aminoantipyrine} + \text{Phenol} \xrightarrow{\text{POD}} \text{Quinoneimine} + 4H_2O$$

Reagents

A) GOD-POD reagent

1. Phosphate buffer (pH 7.5) 250 mmol/L
2. Phenol 5 mmol/L
3. 4-aminoantipyrine 0.5 mmol/L
4. GOD ≥ 10 KU/L
5. POD ≥ I KU/L

B) Glucose Standard: 100 mg/ dl

Method: 10µl of serum aliquots were pipetted in 10×75 mm tubes to which 1mL of glucose reagent was added. The tubes were then gently shaken and incubated at 37 ^{0}C for 10 min and the absorbance (OD) was read at 505 nm using spectrophotometer (PD-303). Along with the sample, separate test tube containing 10µl of glucose standard and 1ml of reagent was identically processed. Another test tube containing only 1ml of reagent served as the reagent blank and was also processed in the same manner.

Protocol for Glucose Estimation

Reagent/ Solution (mL)	Blank (µl)	Standard (µl)	Test (µl)
Glucose Standard	-	10	-
Sample	-	-	10
Reagent	1000	1000	10000

A. Incubate at 37 ^{0}C for 10 min
B. Absorbance is read at 546 nm against reagent blank.

Calculation

$$\text{Concentration of glucose (mg/dl)} = \frac{\text{OD Unknown}}{\text{OD Standard}} \times \text{Concentration of standard}$$

Plasma total protein (g/ dl)

Plasma total protein was estimated by Biuret method (Rifai *et al.*, 1999) using standard kits from serum previously stored at -20 ^{0}C. The details of the method are given below:

Principle: Proteins together with copper ions form a violet blue colour complex in alkaline solution. The absorbance (OD) of the colour is directly proportional to the concentration of TP in the specimen.

$$\text{Protein} + Cu^{++} \longrightarrow \text{Colour complex}$$

Reagents

A) Biuret reagent

1. Sodium hydroxide 500 mmol/L
2. Potassium sodium tartarate 35 mmol/L
3. Potassium iodide 30 mmol/L
4. Copper sulphate 10 mmol/L

B) Protein standard: 6 g/dl

Method: 20 µl of serum was taken in a test tube and 1mL of biuret reagent was added. The contents were mixed and the test tubes were kept at 37℃ for 5 min during which violet-blue colour developed. Absorbance (OD) was then recorded with the help of spectrophotometer (PD-303) at 546 nm. In addition to this, two separate test tubes, one containing 20 µl of standard protein of known strength (6 g/dl) and 1 mL of biuret reagent and the other containing only 1mL of the reagent were processed in the same manner. The latter served as reagent blank.

Protocol for Total Protein estimation

Reagent/ Solution (mL)	Blank (µl)	Standard (µl)	Test (µl)
Protein Standard	-	20	-
Sample	-	-	20
Reagent	1000	10000	10000

A. Incubate at 37 ^{0}C for 5 min
B. Absorbance is read at 546 nm against reagent blank.

3.6.3 Plasma cholesterol (mg/dl)

Method: Cholesterol oxidase-Peroxide/CHOD-POD (Antunovic *et al.*, 2004).

Principle: Determination of cholesterol after enzymatic hydrolysis and oxidation. The colorimetric indicator is quinoneimine which is generated from 4-aminoantipyrine and phenol by hydrogen peroxide under the catalytic action of peroxidase (Trinders reaction).

Cholesterol ester + H_2O ⟶ Cholesterol + Fatty acids
Cholesterol + O_2 ⟶ Cholestrol-3-one + H_2O_2
$2H_20_2$ + 4-Aminoantipyrine + Phenol ⟶ Quinoneimine + $4H_2O$

Method	Endpoint
Slope of reaction	Increasing
Wavelength	546 nm
Flow cell temperature	37℃
Sample	Plasma
Sample volume	10 µl
Reagent volume	1000 µl
Incubation	10 min at 37ºC
Standard concentration	200 mg/dl
Unit	mg/dl
Linearity	600 mg/dl
Normal range	110-200 mg/dl

Test procedure

Pipette into test tubes	Blank (µl)	Standard (µl)	Test (µl)
Protein Standard	-	10	-
Sample	-	-	10
Reagent	1000	1000	1000

3.6.4 Plasma triglycerides (mg/dl)

Method: Glycerol-3-phosphate-oxidase-Peroxidase/GPO-POD (Nazifi *et al.*, 2002).

Principle: Determination of triglycerides after enzymatic splitting with lipoprotein lipase. Indicator is quinoneimine, which is generated from 4-chlorophenol by hydrogen peroxide under the catalytic action of peroxidase.

Triglycerides + H_2O ⟶ Glycerol + Fatty acids
Glycerol + ATP ⟶ Glycerol-3-phosphate + ADP
Glycerol-3-phosphate + O_2 ⟶ Dihydroxyacetone-phosphate + H_2O_2
H_2O_2 + 4-aminoantipyrine + p-Cholorophenol ⟶ Quinoneimine + HCL + $4H_2O$

Method	End point
Slope of reaction	Increasing
Wavelength	546 nm
Flow cell Temperature	37℃
Sample	Plasma
Sample volume	10 µl
Reagent volume	1000 µl
Incubation	10 min at 37℃
Standard concentration	200 mg/dl
Unit	mg/dl
Linearity	1000 mg/dl
Normal range	0-150 mg/dl

Test procedure

Pipette into test tubes	Blank (µl)	Standard (µl)	Test (µl)
Standard	-	10	-
Sample	-	-	10
Reagent	1000	1000	1000

Mix well, and incubate for 10 min at 37 ºC. Read absorbance at 546 nm against reagent blank.

Results and Discussion

Plasma glucose (mg/dl) and protein. (g/dl)

Forthnightly plasma glucose levels in does during peri-parturient period have been presented in Table 2. There was a significant (p<0.05) higher plasma glucose (mg/dl) concentration in T2,T3 and T4 as compared to T1(control) treatment group at kidding and after kidding however there was no significant difference between T2, T3 and T4 treatment group moreever there was no significant difference in different treatment group before kidding at first forthnightly before kidding plasma glucse (mg/dl) concentration in different treatment groups were 62.75±2.22 ,63.28±2.18, 61.95±2.13 and 63.28±1.96 for T1, T2, T3 and T4 respectively. At kidding highest plasma glucose (mg/dl) concentration was highest observed in T3 as 60.78±1.49 mg/dl and lowest in T1 as 53.16±2.65 mg/dl. At last forthnightly after kidding in T3 treatment group as 58.50±1.14 mg/dl and lowest in T1 as 52.00±2.64. Forthnightly plasma total protein (g/dl) concentration in does during peri-parturient period have been presented in Table 3 there was a significant (p<0.05) higher plasma protein (g/dl) concentration in T2, T3 and T4 as compared to T1(control) treatment group at kidding and after kidding however there was no significant difference between T2, T3 and T4 treatment group. Moreever there was no significant difference in different treatment group before kidding. At first forthnightly before kidding plasma protein (g/dl) concentration in different treatment groups were 7.08±0.52, 7.13±0.55, 6.68±0.26 and 7.11±0.54for T1, T2, T3 and T4 respectively. At kidding highest plasma protein (g/dl) concentration was observed in T4 as 6.50±0.32 g/dl and lowest in T1 as 4.25±0.34 g/dl at last forthnightly after kidding in T4 6.11±0.38 treatment group as 58.50±1.14 mg/dl and lowest in T1 as 4.00±0.28. These findings are in agreement with earlier reports by Murniati *et al.* (2013) [9] and

Khatun *et al.* (2010) [5] who found that significantly higher values of glucose in higher concentrate supplemented groups. El-Sheriff (2009) [3] reported that decreasing trend in plasma glucose after kidding can be explained on the basis of heavy demand of glucose for lactose synthesis (constituent of milk) during this period. Sahu *et al*. (2014) reported that significant differences ($P<0.05$) were observed for serum glucose in concentrate supplemented group as (53.115 ± 2.089 mg/dl) as to compared non-supplemented (46.730 ± 0.815 mg/dl). Karapehlivan *et al.* (2007) [4]; Mohri *et al*. (2007) [8] and Roubies *et al*. (2006) [11] reported the decline in plasma total protein particularly γ-globulin after kidding, is due to its removal from the blood stream in order to support mammary secretion. In contrast to present study Sahu *et al.* (2014) reported that non-significant results were observed for total protein, albumin, globulin and A/G ratio between the concentrate supplemented groups prior to kidding. Özsoy *et al.* (2013) [10] reported that blood plasma protein levels were not affected from concentrate supplementation. Luna-Orozco *et al.* (2015) [6] reported plasma concentration total protein were not affected ($P>0.05$) by concentrate supplementation.

Table 2: Mean (±SE) Plasma glucose (mg/dl) of periparturient does supplemented extra concentrate with or without probiotics

		Different treatment groups			
Stage	**Days**	**T1(control)**	**T2**	**T3**	**T4**
Pre partum	-28	62.75±2.22	63.28±2.18	61.95±2.13	63.28±1.96
	-14	62.98±2.09	63.45±2.14	62.11±2.05	63.50±1.87
Kidding	0	53.16±2.65^a	60.16±1.11^b	60.78±1.49^b	59.66±0.77^b
Post partum	14	51.83±0.65^a	58.50±1.11^b	59.33±0.49^b	59.16±1.77^b
	28	52.00±2.64^a	58.33±1.08^b	58.66±1.14^b	58.50±1.47^b

Mean with different superscript in a row differ significantly

Table 3: Mean (±SE) Plasma total protein (g/dl) of periparturient does supplemented extra concentrate with or without probiotics

	Days	**T1(control)**	**T2**	**T3**	**T4**
Pre partum	-28	7.08±0.52	7.13±0.55	6.68±0.26	7.11±0.54
	-14	6.58±0.52	6.95±0.57	6.43±0.34	6.78±0.40
Kidding	0	4.25±0.34^a	6.13±0.55^b	6.38±0.41^b	6.50±0.32^b
Post partum	14	4.25±0.35^a	6.10±0.61^b	6.13±0.38^b	6.38±0.31^b
	28	4.00±0.28^a	6.01±0.61^b	5.62±0.38^b	6.11±0.38^b

Mean with different superscript in a row differ significantly

Forthnightly Plasma triglyceride and Plasma cholesterol (mg/dl)

Forthnightly plasma triglyceride (mg/dl) level in peri-parturient does under different treatment group has been presented in Table 4 At first fortnightly before kidding plasma triglyceride concentration (mg/dl) in different treatment groups were 25±1.40, 27±1.06, 27.33±0.91 and 24.83±1.32 for T1, T2, T3 and T4 respectively. At last forthnightly after kidding plasma triglyceride concentration (mg/dl) in different treatment groups were 24.66±1.56, 26±0.81, 26.33±0.88 and 23.66±1.30 for T1, T2, T3 and T4 respectively. Forthnightly plasma cholesterol (mg/dl) concentration in peri-parturient does under different treatment group has been presented in Table 5. No significant difference was found in different treatment group during study period. At first forthnightly plasma cholesterol (mg/dl) concentration were75.16±1.04 76.33±1.33 76.83±1.22 75.83±1.70 for T1, T2, T3 and T4 respectively. At the end of trail plasma cholesterol concentration (mg/dl) was 74.00±0.93, 75.16±1.55, 75.66±1.42, 74.66±1.7. For T1, T2, T3 and T4 respectively. The present study is in agreement with Skotnicka *et al*. (2011) [13] who reported that one week before delivery triglyceride and TCH concentrations (0.32 ± 0.16 and 1.65 ± 0.42 mmol/l) were significantly increased as compared to non-pregnant goats (0.15±0.05 and 1.38±0.19 mmol/l). But after delivery concentrations of triglyceride and TCH, decreased significantly. Luna-Orozco *et al.* (2015) [6] found that plasma concentrations of cholesterol were not affected ($P > 0.05$) by concentrate supplementation. Özsoy *et al.* 2013 [10] reported that total triglyceride and total cholesterol were not affected from concentrate supplementation.

Table 4: Mean (±SE) of forthnightly Plasma TG (mg/dl) of periparturient does supplemented extra concentrate with or without probiotics

		Different treatment groups			
Stage	**Days**	**T1(control)**	**T2**	**T3**	**T4**
Pre partum	-28	25.50±1.40	27.00±1.06	27.33±0.91	24.83±1.32
	-14	25.33±1.30	26.83±0.94	27.16±0.79	24.66±1.20
Kidding	0	25.00±1.50	26.50±0.84	26.83±0.94	24.33±1.47
Post-partum	14	24.83±1.49	26.16±0.74	26.50±0.84	23.83±1.32
	28	24.66±1.56	26.00±0.81	26.33±0.88	23.66±1.30

No significant differences has been observed in plasma TG among different treatment gr

Table 5: Mean (±SE) of forthnightly Plasma cholesterol (mg/dl) of periparturient does supplemented extra concentrate with or without probiotics

		Different treatment groups			
	Days	**T1(control)**	**T2**	**T3**	**T4**
Pre partum	-28	75.16±1.04	76.33±1.33	76.83±1.22	75.83±1.70
	-14	74.83±1.19	76.00±1.29	76.50±1.17	75.50±1.60
Kidding	0	74.50±1.17	75.66±1.60	76.16±1.49	75.16±1.85
Post-partum	14	74.16±0.94	75.33±1.62	75.83±1.49	74.83±1.77
	28	74.00±0.93	75.16±1.55	75.66±1.42	74.66±1.70

No significant differences has been observed in plasma cholesterol among different treatment groups

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