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RESEARCH ARTICLE

Behavioral and Metabolic Response to a Low Dose Administration of Bacterial Endotoxin for Early Detection of Illness in Goats

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ABSTRACT

Eighteen indigenous female goats of 7-8 months of age with average BW 9.8±1.04 kg (mean±SE) were randomly distributed into three equal groups (CON, LET and HET). Bacterial endotoxin (*Escherichia coli* O55:B5) was injected intravenously to treatment group animals at low (LET; 0.2µg/kg BW) and high (HET; 0.4µg/kg BW) doses while the animals of control (CON) group were administered an equivalent volume of normal saline. Heart rate, rectal temperature, feed and water intake and behavioral changes were studied as the main indicators of the response together with selected blood metabolites. Both the endotoxin treated-groups showed almost similar response of depression and lethargy followed by biphasic shivering, increased ($P<0.05$) rectal temperature with decrease activity, feed ($P=0.054$) and water ($P<0.05$) consumption. No significant differences were observed in studied behavioral, heart rate, hematological (Hb and Hct), serum protein and activity of ALT. Plasma glucose level, on the other hand, was reduced at 3h post-injection in both LET and HET as compared to CON. The serum activity of AST exhibited a significant ($P<0.05$) increase in HET group as compared to LET or CON. Low doses of endotoxin (~0.4 µg/kg BW) in goats mimic low concentration of circulating bacterial endotoxin, which could be ascertained from the associated behavioral and febrile responses along with changes in selected blood metabolites.

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INTRODUCTION

Gram-negative bacterial infections such as *Escherichia coli,* Salmonella, Pseudomonas, Proteus and others tend to occur frequently in farm animals. Infections by these organisms result in release of endotoxin from outer bacterial membrane into the host's internal environment which is responsible for some of the most common diseases in farm animals such as enteritis, endometritis, mastitis, etc. Animals fight against the illness by using their entire potent ability of immune response with a consistent pattern of behavioral changes, including reduced feeding and social behavior and increased rest (Johnson, 2002; Dantzer and Kelley, 2007; Yee and Prendergast, 2012; Hussain *et al*., 2012). These behavioral changes occur simultaneously with physiological changes and are adaptive responses helping the animals for efficiently combating against infectious disease (Owen-Ashley *et al.*, 2006; Johnson, 2002). Understanding of these behaviors in a better way helps improving the early detection of illness, restricting the spread of disease in the flock and initiating treatment at the earliest for better result.

Experimental administration of endotoxin, is a well-characterized and widely accepted model of bacterial infection (Stiggera *et al*., 2013) and are mainly limited to laboratory animals which too commonly relates to infectious process and their mechanism. However, there are only a few studies which successfully explored the issues involved in the illness behavior mainly because of technical limitation of assessing the behavioral changes in details in laboratory animals. Research examining behavior responses to low doses of endotoxin on large mammalian species are very few, and mainly include swine (Johnson and von Borell, 1994; Wright *et al*., 2000), dairy calves (Borderas *et al.*, 2008) and primates (Willette *et al*., 2007). There are no studies documenting together the behavior and metabolic response to induced sickness in indigenous goat, an important farm animal of India. Thus for better understanding the early behavioral response of goats to mild illness, we examined the effect of low doses of endotoxin on heart rate, body temperature, feed and water intake and behavioral changes accompanied the presence of fever together with few relevant blood metabolites.

MATERIALS AND METHODS

Animals and experimental design: Eighteen indigenous female goats of 7-8 months of age with mean BW 9.8±1.04 kg were randomly distributed into three equal groups (CON, LET and HET). The animals were offered a nutritionally balanced diet comprised of concentrate mixture and wheat straw. After a week of adaptation, bacterial endotoxin (*Escherichia coli* O55:B5, L2880; Sigma-Aldrich, St. Louis, MO) prepared in sterile pyrogen-free normal saline was injected intravenously to treatment group animals at low (LET; 0.2µg/kg BW) and high (HET; 0.4µg/kg BW) doses while the animals of control (CON) group were administered an equivalent volume of normal saline.

Physiological and behavioral measures: The rectal temperature (RT) and heart rate (HR) were recorded on each goat before and at 3 and 24h of endotoxin injection. Video camera recording was done to record the behavior of each animal between 1.5 to 4.5h post-injection. The feed intake pattern was monitored by weighing residue at 4.5h and 24h after the injection. Water intake was also recorded for each animals offered twice at 1.5h and 4.5h post-injection.

Blood sampling and analysis: Venous blood samples were collected with and without anticoagulant at 0 and 3h after endotoxin administration. The anticoagulated blood was used for estimation of Hb and Hct by cyanmethemo-globin and microhaematocrit method, respectively. Plasma was used for glucose and serum was used for analysis of total protein, albumin, globulin, ALT and AST using commercially available kits (Span Diagnostics Ltd., India).

Statistical analysis: Results obtained from the study were subjected to one way ANOVA using statistical software SPSS v.20.

RESULTS

Animal response and physiological changes: Animals treated with endotoxin exhibited different behavioral changes post-inoculation in comparison to the control animals. There was no effect of dose of endotoxin and both the treatment groups showed almost similar responses. The change of behavior sign started to appear in about 40 to 60 mins after endotoxin administration which became stronger with time and then finally disappeared recovering to normal state. The behavior sign of depression, lethargy, sleepy and uninterested in surrounding were observed at first followed by biphasic shivering with muscles twitching which appeared to be stronger in hind legs. Few animals in the treatment groups showed mild diarrhea. The affected animals stood still with no obvious movement. These behavior changes were common to both the treatment groups, although the response strength varied from one individual to another. Some animals responded very early with mild behavior changes whereas others took time to respond.

Endotoxin treated animals showed increased rectal temperature with reduced activity and feed consumption. The time course changes of physiological variables like HR and RT after endotoxin injection are listed in Table 1. There was no statistical difference in the HR of the animals among the three groups. Administration of endotoxin increased ($P<0.05$) the RT in LET and HET groups by 2.08 and 1.97^{o}F, respectively, after 3h, whereas the increase was only 0.92^{o}F in the CON group animals injected with normal saline. However, no impact of the endotoxin could be observed when RT was recorded after 24h of endotoxin administration.

Behavioral attributes and feed and water consumption: Mean value of some important behaviors (standing, lying, urination and defecation) during 1.5h before and after peak RT and intake of feed and water during 4.5h after normal saline or endotoxin injections are shown in Table 2. Although the treated animals evidently spent more time lying down, there was statistically no difference ($P>0.05$) among the groups in the time spent for standing or lying. Likewise, the frequency of urination was comparatively higher in the treated groups of animals, although non-significantly. However, there was no difference in the frequency of defecation among the three groups of goats. The treatment with endotoxin resulted in a significant ($P<0.05$) reduction in water consumption by the animals under the LET and HET groups as compared to the CON; there was however no difference between the two treatment groups. There was lower intake of concentrate supplement by both the treatment groups compared to the CON. However, the intake of straw remained similar ($P>0.05$) among the three groups. Consequently, the total feed consumption over the period of 4.5h was reduced in both the endotoxin-treated groups. The rate of feed consumption also showed lower values in the LET and HET vis-à-vis the CON goats. The animals preferred to consume concentrate in comparison to wheat straw to meet their nutritional requirement. Few animal were found to eat wheat straw together with concentrate but most of them started consuming wheat straw only after finishing the concentrate part of the total feed offered. This kind of selective feeding was observed irrespective of endotoxin treatment. There were, however, no differences observed in feed intake after 24h of endotoxin or normal saline injection.

Hematology and blood metabolites: Data on the blood biochemical parameters are presented in Table 3. There were no differences among the groups in the 0h values of any of the blood parameters. No significant differences were observed in studied hematological (Hb and Hct) variables among the three groups at 3h post-injection. The serum levels of total protein, albumin, globulin and A:G ratio remained similar ($P>0.05$) among the three groups when measured after 3h of endotoxin injection. The 3h-plasma glucose level, on the other hand, showed a significant ($P<0.05$) reduction in both the endotoxin-administered groups as compared to the control. There

Table 1: Variations in heart rate and rectal temperature of goats exposed to low (LET; 0.2µg/kg BW) and high (HET; 0.4µg/kg BW) dose of endotoxin

Parameters	Treatment groups			SEM	P-Value
	CON	LET	HET		
Heart rate (beats/min)					
0h	78.67	80.00	76.67	3.10	0.751
3h	93.33	101.33	99.00	4.08	0.385
Changes (0-3h)	14.67	21.33	22.33	3.26	0.228
24h	79.33	82.00	79.00	2.59	0.675
Rectal temperature (°F)					
0h	102.20	102.28	102.72	0.24	0.280
3h	103.12^a	104.37^b	104.68^b	0.17	<0.001
Changes (0-3h)	0.92^a	2.08^b	1.97^b	0.21	0.002
24h	102.08	102.33	102.60	0.17	0.139

^{ab}Values in a row bearing different superscripts differed significantly (P<0.05).

Table 2: Behavioral attributes† and feed consumption of goats exposed to low (LET; 0.2µg/kg BW) and high (HET; 0.4µg/kg BW) dose of endotoxin

Parameters	Treatment groups			SEM	P Value
	CON	LET	HET		
Standing (min)	147	133	138	10.82	0.658
Lying (min)	33	47	42	10.82	0.658
Urination (times)	0.50	1.17	0.83	0.41	0.532
Defecation (times)	3.17	3.50	3.33	0.76	0.953
Water intake (ml)	689.83^a	327.00^b	412.12^b	83.63	0.020
Feed intake (initial 4.5h)					
Concentrate (g)	234.17	153.00	146.67	25.91	0.055
Wheat straw (g)	41.67	21.67	23.33	9.13	0.260
Total (g)	275.80	174.65	169.93	31.65	0.054
Rate of feed consumption (g/min)	1.02^a	0.65^b	0.63^b	0.12	0.054
Total feed intake (24h; g)‡	402.47	344.65	326.60	36.07	0.327

^{ab}Values in a row bearing different superscripts differed significantly (P<0.05); †During 90min before and after peak rectal temperature (at 3h post-inoculation); ‡Total feed intake after 24h of normal saline or endotoxin injection.

Table 3: Selected hematological and blood metabolites† of goats exposed to low (LET; 0.2µg/kg BW) and high (HET; 0.4µg/kg BW) dose of endotoxin before and at 3h (peak rectal temperature) after injection

Parameters	Hour post-injection	Treatment groups			SEM	P Value
		CON	LET	HET		
Haematocrit (%)	0h	30.50	27.83	27.83	1.04	0.146
	3h	27.83	26.17	27.67	1.18	0.558
Hemoglobin (g/dl)	0h	14.41	12.89	13.44	0.56	0.189
	3h	13.88	13.03	13.31	0.70	0.688
Glucose (mg/dl)	0h	66.39	64.30	62.76	3.22	0.732
	3h	82.92^a	66.81^b	62.06^b	4.57	0.014
Total protein (g/dl)	0h	7.31	6.71	7.05	0.47	0.674
	3h	6.25	6.28	6.56	0.27	0.676
Albumin (g/dl)	0h	3.28	3.30	3.13	0.14	0.665
	3h	3.24	3.02	3.05	0.16	0.573
Globulin (g/dl)	0h	4.03	3.40	3.92	0.44	0.565
	3h	3.01	3.26	3.51	0.23	0.343
A:G ratio	0h	1.06	0.99	0.83	0.20	0.695
	3h	1.11	0.93	0.91	0.09	0.243
ALT (IU/L)	0h	44.51	45.76	48.32	4.74	0.847
	3h	89.30	96.86	117.48	6.97	0.296
AST (IU/L)	0h	87.66	92.15	99.17	12.70	0.515
	3h	102.73^a	114.84^a	173.41^b	12.20	0.002

^{ab}Values in a row bearing different superscripts differed significantly (P<0.05).

was no variation in the serum activity of ALT in either periods of measurement. However, there was a significant (P<0.05) increase observed in serum activity of AST in HET group, measured at 3h post-endotoxin treatment, as compared to LET or CON.

DISCUSSION

Behavior changes were observed following the endotoxin administration in animals of both the treatment groups. Mild doses of endotoxin result in shivering due to parasympathetic nervous system excitation accompanied by febrile response, an early indicator of development of sickness. There was no significant change in the heart rate upon endotoxin administration recorded in our study. Possibly the responses of the goats to the handling involved in measuring HR might have masked the supposedly effects of endotoxin on the HR. An increase was recorded in rectal temperature in the endotoxin-treated groups but no differences were found between high and low doses of endotoxin. This could be possibly because of the fact that both doses were already mild for goats. The current observations on the effects of endotoxin on rectal temperature are in agreement with those reported previously for sheep (Yates *et al*., 2011) and goats (Takeuchi *et al*., 1995).

There are varied reports describing the impact of endotoxin administration on the time spent on standing and lying. A previous research has indicated an increase in the time spent lying down after endotoxin administration (Johnson and von Borell, 1994); however, there are reports to the contrary (Takeuchi *et al*., 1995). We found no change in overall time spent standing or lying during the period of 1.5h before and after the peak rectal temperature. The frequency of urination and defecation were almost similar among the groups. No difference was found in defecation frequency which reflects no disturbance in the motility pattern of gastrointestinal tract.

A decrease in feed intake after endotoxin administration was observed in most of the earlier studies (Wright *et al*., 2000; Kim *et al*., 2007; Asarian and Langhans, 2010). Takeuchi *et al*. (1995) found a significant decrease of feed and water intake and time spent for feeding with endotoxin injections during an 11h-observation period in goats. Similar observations were recorded in our experiment, where endotoxin treated animals, irrespective of the dose, showed similar trends of decrease feed and water consumption with a demonstrated preference for concentrate than wheat straw during the period leading to the peak fever. This preference for concentrate might be due to its higher nutritional value as there are reports that sickness induced no change in the ingestion of nutritious food (Aubert and Dantzer, 2005). The lack of interest in feed consumption as evident from the lower rate of feed consumption (Table 2) due to endotoxin administration was not observed at 24h after injection. This, in turn, is indicative of normalization of the adverse effects of endotoxin with time and could have been caused by compensatory increase in feed consumption beyond the acute phase. In fact, a deeper look into the feed consumption pattern indicates that the average feed intake in the LET and HET groups were 63.3 and 61.6% of the CON value within first 4.5h of endotoxin injection. However, when compared after 24h, the respective values are 85.6 and 81.1% of the CON value (100%). As a result, amount of the feed consumed between 5.5 and 24h post-administration was more in LET (170.0g; 134.2%) and HET (156.7g; 123.7%) as compared to CON (126.7g; 100%). Clearly, there was a compensatory increase in feed consumption in the endotoxin-treated groups as time elapsed. In contrast, Steiger *et al*. (1999) found a significant decrease in food intake at both 4h and 24h after infusing heifers with endotoxin at 2µg/kg BW. This difference could be because of prolong infusion period of endotoxin.

Endotoxin administration at the dose used in the experiments showed no significant alterations in the hematological profile in treatment groups in comparison to the control. The observed Hb and Hct level was close to the normal range as reported for goats by Latimer *et al*. (2003). There were little changes seen in serum levels of total protein, albumin, globulin, A:G ratio in blood collected before and at 3 h after endotoxin administration suggesting little or no adverse effect of endotoxin administration at the delivered doses in the present study. Plasma levels of glucose normally increase post-prandially because of the digestion of dietary carbohydrates, and the same was evident in the control group. On the similar line, the reduced ($P<0.05$) glucose level observed at 3h post-administration in the treatment groups correlated well with the observed decline ($P=0.055$) in concentrate consumption. The increase in the serum levels of both the aminotransferases (ALT and AST) at 3h post-injection compared to their respective 0h values, observed across the groups, could be related to the post-prandial increase of systemic origin. However, higher ($P<0.05$) values of AST in HET as compared to LET or CON groups indicated that administration of endotoxin at 0.4µg/kg BW did affect the nitrogen (amino acid) metabolism at the hepatic level.

Endotoxin administration has often been used to study sickness behavior (Dantzer, 2009; de Paiva *et al*., 2010; Ribeiro *et al*., 2013), but there are limitations with using endotoxin to model behavioral responses to illness. The major problem includes the difficulty in establishing the time of beginning of illness, and the physiological state or processes associated with the progression of the disease under natural condition could be very different from that observed under controlled experimental conditions. However, this difficulty was theorized to be reduced or eliminated when using low doses of endotoxin in conjunction with assessment of hematological and blood metabolic profile. The main disadvantage of the endotoxin model in the present experiment is the abrupt development of clinical signs and its persistence for a very short duration compared with naturally acquired diseases. One of the reasons for this could be the low doses of endotoxin (~0.4µg/kg BW) used in the present study. Despite this limitation, use of the designated doses of endotoxin did induce febrile response in the goats accompanying behavioral changes especially those related to activity, feed and water consumption. This taken together the observed changes in blood glucose level and AST activity are expected help understand the behavioral correlates of the beginning of illness, their detection and treatment at early stage in goats.

Conclusion: Low dose of endotoxin (~0.4µg/kg BW) in goats mimic low concentration of circulating bacterial endotoxin and could be correlated with changes associated with the beginning of some infectious diseases. The lack of an incubation period, the short duration of the effect, and individual differences in sensitivity to endotoxin are factors that must be considered as limitations of this model for early detection of illness. Nonetheless, the observed febrile and behavioral responses along with changes in select blood metabolites like glucose and AST could be correlated to the impeding infection in susceptible indigenous goat population.

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