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Effect of Yeast Origin Mannan Oligosaccharide and β-Glucan Prebiotic Supplementation on Disease Susceptibility and Growth Performance of Preweaned Goat Kids

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ABSTRACT

Background: A study was conducted to evaluate the effect of prebiotic containing yeast origin mannan oligosaccharide and β-glucan (Agrimos®) on immunity, disease susceptibility and growth performance of newborn goat kids.

Methods: The prebiotic was directly fed to kids from day 03 to day 30 of life @ 100 mg/kg body weight/day.

Result: Results revealed significant reduction in lipopolysaccharide induced release of cytokines- TNF-α as well as IL-10 in the supplemented group when compared to the unsupplemented group. The group treated with prebiotic, exhibited enhanced resistance to infections during the initial two months of life. The protective effect of the prebiotic against cumulative disease incidence persisted as long as it was regularly administered, but gradually waned off after the cessation of feeding. Prebiotic supplementation provided substantial protection against infectious diseases, reducing morbidity and proving effective against septic insults in goat kids. Given that neonatal goat kids are born agammaglobulinemic with immature innate and adaptive immune systems, prebiotic supplementation could serve as a beneficial approach to enhance their health, boost their immunity and promote their overall metabolic activity until their immune system becomes fully functional.

Key words: β-Glucan, Goat Kid, Jamunapari, Mannan oligosaccharide, Morbidity, Prebiotic, Sepsis.

INTRODUCTION

In Indian subcontinent, the goats are reared primarily for its meat, as there is no religious taboo attached to goat meat. Farmers and entrepreneurs have sensed an opportunity in this and have responded by adopting intensive goat farming system. However, high morbidity and mortality due to infectious diseases have emerged as significant roadblock to profitability of goat enterprise. Of all the age groups, neonatal goat kids are most susceptible to infectious diseases particularly diarrhoea and pneumonia (Sharma *et al.,* 2022; Srivastava *et al.,* 2022). Various measures have been adopted to reduce the disease incidences in newborn farm animals including in-feed antibiotic growth promoters (AGP). Use of AGP has been subjected to criticism recently due to increasing awareness about antibiotic resistance (Sharma *et al.,* 2022; Singh *et al.,* 2022; Srivastava and Mondal, 2016) and drug residues in animal products. After that AGPs have been completely banned by the European Union since 2006 (https://ec.europa.eu/commission/presscorner/detail/en/ip_05_1687; Millet and Maertens, 2011).

There is pressing need to evaluate alternatives to replace in-feed antibiotics to improve health and growth of farm animals. In this regard, the preferred approach is evaluating the effect of prebiotic and probiotic supplementation on immune status of farm animal neonates. Among prebiotics, digestion resistant oligosaccharides, mainly mannan oligosaccharide (MOS) are reported to positively affect the gut health and systemic immunity in ruminant neonates (Alves Costa *et al.,* 2019).

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However, there is no report on effects of prebiotics on health and growth performance of small ruminants particularly goat kids. In light of the above facts, the present study was designed to evaluate the effect of prebiotic containing MOS and β-glucan (Agrimos®) on immunity, disease susceptibility and growth of newborn goat kids.

MATERIALS AND METHODS

The study was conducted at the Jamunapari breed unit of Indian Council of Agricultural Research - Central Institute for Research on Goats (ICAR - CIRG), Mathura, India from 2019-2023. The institute is located at an altitude of 163.4

meters above mean sea level at latitude of 27.10°N and longitude of 77.9°E.

Goats were managed in a semi-intensive system with common feed source and grazing area. All the animals were housed in well-ventilated sheds having facility for individual feeding and watering. A veterinarian monitored health status of all the animals daily. All the animals were regularly dewormed and vaccinated as per the standard protocol (https://cirg.res.in/assets/downloads/hcalender 18. pdf; Srivastava *et al.,* 2022).

Twenty-four healthy full term goat kids immediately after birth were randomly but equally allocated to 02 groups- *viz*. control (C) and prebiotic treated (P). The kids were assisted to suckle the dam for fixed duration within ½ hour after birth. Kids were helped to suckle a second time after 8 hours of first feeding.

Supplementation was started on day 03 of life (nothing was given except colostrum for the first 48 hours as it might interfere with assimilation of maternal immunoglobulins) and given daily upto 30 days of life. Kids of group P were orally given yeast origin prebiotic containing mannan oligosaccharide and beta-glucan suspended in distilled water (Agrimos® provided by Lallemand Animal Nutrition, India) @ 100 mg/kg body weight/day whereas kids of group C were given only distilled water.

Six kids from both group (C and P) at 2 weeks of age were subjected to experimental sepsis by sub-lethal bolus intravenous injection of purified *Escherichia coli* O111:B4 lipopolysaccharide (Sigma® L 3024) @ 200 ng/Kg body weight (Takeuchi *et al.,* 1995). Clinical (heart rate and rectal temperature) and behavioral (demeanor and suckling) parameters were recorded after one hour of lipopoly saccharide (LPS) injection. Parameters of six healthy kids of similar age group of same breed were also recorded to serve as control (Group: No Prebiotic and No LPS) (Table 1). Two ml blood was collected in heparinized vacutainer (Greiner Bio-One GmbH, Austria) after 04 hours of LPS administration. Hematological analysis of blood was done within 01 hour of bleeding using automatic hematology analyzer standardized for goats (Melet Schloesing Laboratoires, France) and plasma was separated and stored at -20°C for estimation of cytokines (TNF-α, IFN-γ and IL-10). Cytokines were measured in plasma using goat specific quantitative ELISA kits (Shanghai Bluegene Biotech co. ltd®, Shanghai, China). Clinical Scoring was performed with some modifications in the procedure used for cattle calves (Gerros *et al.,* 1995).

The attending veterinarian, who was blinded to the experiment, observed health status of kids on daily basis. All disease incidences were recorded (except those attributable to trauma or mis-mothering). Diseased kids were isolated from the group and treated separately.

Goat kid's body weight, chest girth and wither height were recorded on day of birth (DOB/ Day 0) and then fortnightly till 180 days of age to assess growth pattern.

Experimentation protocol of this study was approved by institutional animal ethics committee (IAEC) of ICAR-CIRG, Mathura.

Mean values of the different study parameters were compared by one-way analysis of variance (ANOVA) with post-hoc Tukey HSD test using online statistical calculator (http://astatsa.com/OneWay_Anova_with_TukeyHSD/)

Chi-square (χ^2) test was applied to test association between treatments and disease susceptibility using online statistical calculator (https://www.socscistatistics.com/tests/chisquare/default.aspx) .

RESULTS AND DISCUSSION

Effect on response to induced sepsis

Clinical parameters

The goat kids of group C and P subjected to intravenous LPS had significantly higher rectal temperature and depression score after one hour of injection compared to unexposed kids (Group: No prebiotic and No LPS) (Table 1). Rectal temperature and depression score of kids supplemented with prebiotic (P) was significantly less than that in the supplemented kids (Table 1).

Leukogram

Kids of both the groups developed significant leukopenia after 4 hours of LPS administration compared to healthy kids not exposed to LPS. Greater degree of leukopenia was observed in kids of prebiotic supplemented group (P) compared to those of the control (C) group. However, kids of control (C) group experienced significant lymphopenia and agranulocytosis compared to kids of group P (Table 2).

Cytokine profile

Serum concentration of TNF-α as well as of IL-10 was significantly higher in the kids of group C when compared to the kids of group P (Table 3).

Enteric infections, pneumonia, septicemia and frequently combination of these disease entities are majorly

Table 1: Clinical response of prebiotic supplemented goat kids post 1 hour to intravenous LPS.

Group (N=6)	Heart rate (bpm)	Rectal temperature (°F)	Depression score
C	130.2±3.78	106.0±0.11^{A}	2.33±0.21^{A}
P	145.8±4.55	104±0.34^{B}	1.67±0.21^{B}
No prebiotic and no LPS	134.8±4.87	102.7±0.19^{C}	0.00^{C}

*Values with different alphabets as superscript across the column differ significantly (P<0.05).

C: Control (No prebiotic supplementation), exposed to LPS; P: Prebiotic MOS supplemented, exposed to LPS; No prebiotic and No LPS: No prebiotic supplementation and no exposure to LPS.

Table 2: Leukogram of prebiotic supplemented goat kids to intravenous LPS.

Group (N=6)	TLC (×1000/µl)	Granulocyte (%)	Lymphocyte (%)
C	9.8±0.1959^{A}	79.8±0.7661^{A}	15.8 ±0.7885^{A}
P	4.8±0.4106^{B}	62.7±1.8402^{B}	32.2±1.7423^{B}
No prebiotic and no LPS	12.3±0.9399^{C}	66.4±1.4569^{B}	26.2±1.0279^{C}

*Values with different alphabets as superscript across the column differ significantly (P<0.05).

C: Control (No prebiotic supplementation), exposed to LPS; P: Prebiotic MOS supplemented, exposed to LPS; No prebiotic and No LPS: No prebiotic supplementation and no exposure to LPS.

Table 3: Cytokine profile of prebiotic supplemented goat kids after intravenous LPS.

Group (N=6)	TNF-α (pg/ml)	IL-10 (pg/ml)	IFN-γ (pg/ml)
C	495.96±27.87^{A}	796.06±61.01^{A}	525.71±37.65^{A}
P	381.46±7.83^{B}	601.18±16.65^{B}	503.45±52.81^{A}

*Values with different alphabets as superscript across the column differ significantly (P<0.05).

C: Control (No prebiotic supplementation), exposed to LPS; P: Prebiotic MOS supplemented, exposed to LPS.

responsible for mortality in goat kids and other ruminant neonates (Sharma *et al.,* 2022; Singh *et al.,* 2022; Srivastava *et al.,* 2022, Mishra *et al.,* 2022). Additionally, elevated levels of proinflammatory cytokines - IFN-γ and TNF-α and acute phase proteins - serum amyloid A (SAA) and haptoglobin (Hp) have been recorded in infected diarrhoeic calves (Pourjafar *et al.,* 2011). It is fairly possible that a major proportion of neonatal goat kids' death due to bacterial diarrhea and pneumonia is not only due to dehydration, electrolyte imbalance and hypoxia but inflammatory response to endotoxemia is also an important contributing factor. Even small amount of endotoxin has been reported to cause severe pulmonary injury in sheep and calves (Winkler, 1988).

Sepsis activates an array of immunological pathways leading to surge of pro-inflammatory (*e.g.* IL-12, TNF-α, IL-6) as well as anti-inflammatory/regulatory (IL-10) cytokines. Normally, release of IL-10 increases proportionately to that of pro-inflammatory cytokines. This might be an adaptive response to prevent tissue damage and homeostasis dysregulation due to surge of pro-inflammatory cytokines. Elevated serum concentration of TNF-α, IL-10 and IL-10: TNF-α ratio have been linked to increased severity of sepsis and mortality (Basoglu *et al.,* 2004; Gogos *et al.,* 2000).

Neonates are more susceptible to sepsis than adults resulting in comparatively higher mortality. Their increased susceptibility is mainly due to functional immaturity of innate and adaptive immune system. Studies indicate that in neonates phagocytic function of immune cells is also not fully developed (Cuenca *et al.,* 2013). This downregulation of Th1 type of immune response has been found to occur at signaling level post TLR activation. Of all the toll like receptors, TLR4 is the most important one which is expressed in leukocytes and intestinal epithelium and acts as receptor for lipopolysaccharide (bacterial endotoxin) mediated immune response in case of gram negative enteric infections and sepsis (Chassin *et al.,* 2010).

In the present study, supplementation with prebiotic resulted in significantly lower LPS induced release of cytokines-TNF-α as well as IL-10 compared to unsupplemented goat kids. However, IL-10 to TNF-α ratio was similar among the three groups. In addition, prebiotic supplementation did not affect release of IFN-γ, which was comparable to that in the control group. As discussed previously, if exaggerated release of TNF-α followed by tandem and equivalently exaggerated release of IL-10 is associated with increased mortality in septic animals; results of the present experiment indicate that the prebiotic supplementation might be protective against septic insult in goat kids.

Neonates are extremely vulnerable to infectious diseases. However, early neonatal life is the period when sterile GI tract of neonate is exposed to a multitude of microorganism and food antigens. At this stage, the mucosal and systemic immune system has to learn to 'accept' the beneficial/commensal and 'reject' the detrimental/pathogenic antigenic stimulus - leading to establishment of a healthy GI microbiome. For establishment of early microbiome, the mucosal immune system needs to be in a tolerant state and need not to react with exaggerated inflammation. Th2/Th17 polarized innate immune system might facilitate development of such a tolerant state (Tourneur and Chassin, 2013).

Improper establishment of healthy GI microbiome not only disrupts GI mucosal immune maturation but also allows translocation of pathogenic bacterial into systemic circulation rendering the neonate susceptible to sepsis (Tourneur and Chassin, 2013).

Effect on disease occurrence/ morbidity

Cumulative incidence of acute infectious diseases (enteritis, pneumonia, fever *etc*.) in kids of both the groups was highest in 2nd month of life and least in 3rd month of life. During 1st and 2nd month, higher proportion was observed in control (C) group than that in group P; the difference being statistically significant in 1st month. A sudden spurt in disease incidences (mainly diarrhea) was observed in kids of group P during the 2nd month (just after

Table 4: Cumulative incidence of acute infectious diseases in goat kids up to weaning.

Group	No. of kids	% Disease occurence/morbidity		
		1st month	2nd month	3rd month
C	12	83.33 (10/12)	91.67 (11/12)	0
P	12	25.00 (3/12)	66.67 (8/12)	0
	χ^2	8.22	2.27	-
	p-value	.004	1.01	-

C: Control (No prebiotic supplementation); P: Prebiotic MOS supplemented.

Table 5: Growth pattern of prebiotic supplemented goat kids.

Group	Δ Body weight (kg) in age range (D)			ΔChest girth (cm) in age range (D)			Δ Wither height (cm) in age range (D)		
	0 -30	30 - 90	90 - 180	0 -30	30 - 90	90 - 180	0 -30	30 - 90	90 - 180
C (N=12)	2.57±0.10	6.53±0.36^{A}	2.83±0.40	3.83±0.30	12.1±0.52^{A}	5.08±0.15	4.75±0.28	13.75±0.74^{A}	5.00±0.35
P# (N=10)	2.49±0.10	5.03±0.41^{B}	3.33±0.48	4.20±0.13	10.10±0.48^{B}	5.60±0.50	4.80±0.25	11.10±0.55^{B}	5.70±0.40

*Values (Mean±SEM) with different letters as superscript across column vary significantly (P<0.05).

#Data of 02 kids of group P not included due to illness of dam and resultant poor mothering; C: Control (No prebiotic supplementation); P: Prebiotic MOS supplemented.

cessation of supplementation) which eventually normalized afterwards (Table 4).

In this study, effect of oral supplementation of the prebiotic was also evaluated on susceptibility of goat kids to natural infections. Results indicate that supplementation of the prebiotic confer significant protection against infectious diseases during 1st month of life in goat kids. Whether this protection was mediated through more effective establishment of GI microbiome or favourable priming of innate immune system through other pathways was not assessed in this study. No comparable study in neonatal goat kids could be retrieved.

Effect on growth

No significant difference was observed in weight and height gain of kids among the groups during first 30 days of life. Interestingly, kids of group P grew significantly less than control (C) kids during 30 - 90 days of life. However, growth rate of these prebiotic supplemented kids again accelerated to normal levels during 90-180 days of age (Table 5).

The prebiotic (Agrimos®) used in this study contains two components of yeast (*Saccharomyces cerevisiae*) cell wall - mannan-oligosaccharide (MOS) and β-(1,3 and 1,6)-poly-D-glucose (β-glucan). Extensive studies have been conducted on yeast *S. cerevisiae* and its cell wall derivatives to understand their role as a prebiotic.Consensus is emerging that these substances are immunomodulatory in nature and affect activity of immune system as well as pathogen present in GI tract of the animal. Like other non-digestible oligosaccharides MOS acts as feed source of commensal GI bacteria like bifidobacteria and lactobacilli whereas most pathogenic bacteria including pathogenic *E. coli* are unable to utilize it. In addition, yeast cell wall polysaccharides inhibit attachment of pathogenic bacteria to GI mucosal cells, reducing their colonization and ability to induce disease (Broadway *et al.,* 2015). Dietary supplementation of 0.2% MOS has been shown to significantly reduce plasma concentration of LPS and acute phase proteins in finishing steers (Jin *et al.,* 2014). However, disease susceptibility data of the present study indicates that protective effect of the prebiotic lasted while it was being fed regularly and then gradually waned off after the feeding was ceased.

CONCLUSION

The results of this investigation suggest that the prebiotic supplement, comprised of yeast-derived mannan oligosaccharide and β-glucan (Agrimos®), has a modulatory effect on immunity of neonatal goat kids, which leads to a restrained and more balanced inflammatory response to systemic infections, contributing to an overall enhancement in resistance to infectious diseases during the initial first month of life. Given that neonatal goat kids are born agammaglobulinemic with immature innate and adaptive immune systems, prebiotic supplementation could serve as a beneficial approach to enhance their health by boosting the immune system and improving overall metabolic activity until their immune system becomes fully functional. Given that neonatal goat kids are born agammaglobulinemic with immature innate and adaptive immune systems, prebiotic supplementation could serve as a beneficial approach to enhance their health, boost their immunity and promote their overall metabolic activity until their immune system becomes fully functional.

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Conflict of interest

The authors declare that they have no conflict of interest.

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