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Influence of Amla (*Emblica officinalis* L.) and Fenugreek (*Trigonella foenum-graecum* L.) Supplementation on Haemato-Biochemical Profile in Goat Kids

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ABSTRACT

This study aimed at ascertaining the effect of amla (*Emblica officinalis* L.) fruit powder and fenugreek (*Trigonella foenum-graecum* L.) seed supplementation on haemato-biochemical profile of growing goat kids. For this, 24 healthy uncastrated male kids (age: 4-6 m; mean body weight: 7.49 ± 1.04 kg) were distributed into four groups of six each through a randomized block design. All the kids were fed with a basal diet comprising of concentrate mixture and wheat straw in proportion of 60:40. Additionally, the ration of the kids of group T1 contained no supplement (control), whereas, those of groups T2, T3 and T4 contained 3% amla, 3% fenugreek, and 1.5% amla plus 1.5% fenugreek seeds, respectively on a dry basis. Results of 240 days of feeding trial revealed that haematological parameters measured as haemoglobin and packed cell volume as well as blood metabolites such as glucose, triglycerides, cholesterol, total protein, albumin, globulin, urea, aspartate aminotransferase and alanine aminotransferase did not differ across various dietary treatments. Therefore, it was concluded that supplementation of amla and fenugreek did not influence any of the analysed blood parameters related to energy, protein and fat metabolism, and thus proving safe for use as natural feed additives in goat husbandry.

KEYWORDS: Amla, Blood biochemistry, Fenugreek, Goat nutrition, Natural feed additives

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INTRODUCTION

In India, about 24% of the rural households are engaged in goat farming, and marginal-to-small farmers are the custodians of over 3/4th of the goats maintained on zero-to-low input extensive rearing systems (Singh et al., 2018). Besides balancing the ration for critical nutrients – both macro and micro – feed additives have been suggested to correct any deficiencies, augment the production, profitability and overall sustainability in livestock enterprises, including goats (Michalak et al., 2021). There has been a renewed interest globally among ruminant producers to use plant-based natural feed additives in recent times, and this is particularly true after the ban of in-feed antibiotic growth promoters by European Union (Kholif et al., 2021). Plant-based additives have a range of biologically active compounds (tannins, saponins etc.) that exert multifarious beneficial effects in ruminants (Kumar et al., 2016; Lakhani et al., 2016; Tilahun et al., 2022). Broadly, the effects like favourable modulation of rumen fermentation, increase in nutrient digestion and metabolism leading to a better production response as well as enhancing health status are described for phytogenic additives (Kholif et al., 2021).

Amla or aonla (*Emblica officinalis L. syn. Phyllanthus emblica L.*), also known as Indian gooseberry, is a fruit with immense beneficial bioactive principles like polyphenols, tannins, flavonoids and ascorbic acid, and tannin concentration could be up to 35% in dried fruit powder (Tilahun et al., 2022). Past experiments have demonstrated an improved organic matter

digestibility and rumen fermentation in vitro (Kumar et al., 2016) and maintaining haematological parameters in summer-stressed buffaloes (Lakhani et al., 2016). Fenugreek (*Trigonella foenum-graecum L.*), also known as methi, is known to contain 3-5% saponin (Rejil and Mohini, 2007), and its supplementation enhanced in vitro rumen fermentation variables (Devasena et al., 2010; Kumar et al., 2016), whilst beneficially decreasing methane emission (Rejil and Mohini, 2007; Niu et al., 2021) as well as improving blood constituents in goats (Mir et al., 2013). Furthermore, fenugreek was concluded to be more efficacious in high-concentrate diets for ruminants (Goel et al., 2008). Even though the beneficial effects of both amla and fenugreek are known, a comparative evaluation of both these supplements on the haematological profile of growing goats has not been evaluated. Since it is well known that blood biochemistry acts as an index of health and well-being of animals, and can be used as a tool to evaluate nutritional status (Sharma et al., 2014). Considering these facts in view, the present experiment was designed to ascertain the influence of supplementing amla fruit and fenugreek seed powder, either alone or in combination, on haemato-biochemical variables as relevant to energy, protein and fat metabolism in growing kids.

MATERIAL AND METHODS

Twenty-four healthy uncastrated non-descript male kids (native of Rohilkhand region, UP, India) of about 4-6 months of age were selected from the Sheep and Goat Farm of the institute. These were divided into four groups of six animals each based on comparable body weight (T1: 7.58±1.07 kg, T2: 7.24±1.02 kg, T3: 7.31±1.12 kg and T4: 7.83±0.96 kg) in a randomized block design. All the animals were fed with concentrate mixture and wheat straw in a proportion of 60:40 to meet the nutrient requirements (ICAR, 2013). The concentrate mixture contained crushed maize (30%), soya bean meal (33%), wheat bran (34%), mineral-vitamin mixture (2%) and common salt (1%) on a fresh basis. While the concentrate mixture was offered daily at 9:30AM, wheat straw (particle size: 1-2 cm) was provided *ad libitum* after the complete consumption of concentrate mixture. Additionally, all the kids were fed with seasonally available green forages (maize in kharif and berseem in rabi) twice a week to meet the requirements of vitamin A. Treatments consisted of either no supplement (control: T1), or concentrate mixture containing 3% supplemental amla (T2), 3% methi (T3) and 1.5% each of amla and methi (T4) on the basis of total diet dry matter. All the animals were housed in a well-ventilated shed with ample access to sunshine and managed uniformly, complying with the guidelines of the Institutional Animal Ethics Committee, throughout the feeding trial lasting 240 days.

Blood samples were drawn at an interval of 60 days from all the animals separately through the jugular vein and collected into heparinised vacutainers. The tubes were rotated between palms to ensure proper mixing of blood with anticoagulant. The samples were kept in the ice box and immediately brought to the laboratory. Haematological parameters like haemoglobin (Hb) and packed cell volume (PCV) were analysed immediately in fresh blood sample using an autoblood analyser (Mindray Cell Counter, BC-2800 VET, China). Then, the samples were centrifuged at 3000 rpm for 15 min. to separate plasma, and were stored at -20°C awaiting further analysis. Plasma glucose and blood urea nitrogen (BUN) were estimated using GOD-POD kit (Span Diagnostics Ltd., India). Further, plasma levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein, albumin, triglycerides and cholesterol were estimated using respective analysis kits (Span Diagnostics Ltd., India). Globulin was calculated by the difference between total protein and albumin.

The data were presented as means with a pooled standard error of mean for all parameters. The data analysis was carried out by two-way ANOVA with interaction, and the difference between the means was compared by Duncan's Multiple Range Test using the software package of SPSS (version 20.0, 2012). The statistical significance between the mean values of treatments and periods was established at $P<0.05$.

RESULTS AND DISCUSSION

Blood levels of Hb and PCV did not vary across various diets as well as at different intervals of sampling, except greater (P<0.05) values of PCV were noted at 240 d (Table 1).

Table 1. Haematological and plasma enzyme profile of experimental goat kids

Attribute	Period (days)					Treatment mean	SEM	P value
	0	60	120	180	240			
Haemoglobin (g/dL)								
T1	9.99	10.2	10.2	9.26	9.00	9.71		T=0.164
T2	9.68	9.90	9.87	9.31	9.52	9.66		P=0.965
T3	9.72	10.4	9.78	10.9	10.7	10.3	0.112	T×P=0.575
T4	10.1	9.70	9.72	9.72	9.90	9.83		
Period mean	9.87	10.0	9.88	9.81	9.79			
Packed cell volume (%)								
T1	29.2	30.4	27.4	27.3	31.7	29.2		T=0.473
T2	27.9	29.7	29.5	29.6	30.3	29.4		P=0.050
T3	29.7	29.8	30.4	29.9	30.6	30.1	0.217	T×P=0.461
T4	30.1	29.3	29.2	29.5	30.9	29.8		
Period mean	29.3^{a}	29.8^{ab}	29.1^{a}	29.1^{a}	30.9^{b}			
Aspartate aminotransferase (IU/L)								
T1	70.9	71.7	75.9	71.4	76.4	73.3		T=0.528
T2	69.9	71.2	74.1	74.0	74.7	72.8		P=0.000
T3	72.4	70.4	75.6	75.0	75.7	73.8	0.300	T×P=0.137
T4	72.8	74.3	74.7	75.6	72.0	73.9		
Period mean	71.5^{a}	71.9^{a}	75.1^{b}	74.0^{b}	74.7^{b}			
Alanine aminotransferase (IU/L)								
T1	35.7	34.1	30.5	31.9	32.5	33.0		T=0.584
T2	30.6	37.1	25.3	28.0	31.4	30.5		P=0.224
T3	31.9	38.4	31.0	30.8	29.7	32.4	0.855	T×P=0.916
T4	31.4	34.8	35.5	30.3	36.6	33.7		
Period mean	32.4	36.1	30.6	30.3	32.5			

^{a,b}Means bearing different superscripts in a row differ significantly (P<0.05)
T1: no supplement; T2: 3% amla; T3: 3% fenugreek; T4: 1.5% amla + 1.5% fenugreek

Values for both of these are within the physiological range for goats (Radostits et al., 2007). Although saponins in plant products may cause haemolysis and lower Hb, no such effect was noted in this study. Similarly, Dubey et al. (2012) reported no effect on Hb and haematocrit (PCV) in kids fed leaf meal mixtures rich in plant secondary compounds. Whereas Mir et al. (2013) noted a higher Hb and PCV at 28th day compared to 0th day in goats supplemented with fenugreek seeds, Lakhani et al. (2016) observed a similar result when amla powder was supplemented to summer-stressed buffaloes.

The concentration of AST and ALT in kids did not differ irrespective of dietary treatments and were within the normal physiological range for goats (Kaneko, 1997). Period-wise comparison showed that AST concentration was higher (P<0.05) at 120,

180 and 240 d as compared to 0 and 60 d. While the AST enzyme is present in hepatocytes, red blood cells, cardiac and skeletal muscles, ALT is normally found in hepatocytes only. Our results corroborate closely with Mir et al. (2013), who reported no effect of feeding 3% fenugreek seeds on the levels of AST and ALT in goat kids. Most recently, Tilahun et al. (2022) also obtained similar results when milking cows received 400 g/d of fresh amla fruits.

The blood glucose concentration did not differ due to treatments and periods (Table 2).

Table 2. Blood metabolites of energy, protein and fat metabolism in experimental goat kids

Attribute	Period (days)					Treatment	SEM	P value
	0	60	120	180	240			
Glucose (mg/dL)								
T1	47.2	52.4	52.4	50.4	51.1	50.7	0.635	T=0.542
T2	43.3	52.4	44.1	49.7	51.6	48.2		P=0.157
T3	52.4	53.3	46.3	53.5	45.5	50.2		
T4	53.9	49.1	47.2	51.6	45.2	49.4		
Period mean	49.2	51.8	47.5	51.3	48.3			T×P=0.154
Triglycerides (mg/dL)								
T1	89.4	89.6	96.7	85.9	93.2	91.0	1.281	T=0.502
T2	86.4	91.1	90.4	88.9	95.2	90.4		P=0.622
T3	88.3	82.7	93.9	90.0	89.6	88.9		
T4	97.3	105	85.1	86.1	98.4	94.3		
Period mean	90.3	92.0	91.5	87.8	94.1			T×P=0.512
Cholesterol (mg/dL)								
T1	111	102	122	111	111	111	2.126	T=0.929
T2	99.8	115	105	95.7	122	107		P=0.663
T3	102	105	120	109	109	109		
T4	108	111	107	111	109	109		
Period mean	105	108	114	107	113			T×P=0.841
Total protein (g/dL)								
T1	6.31	6.06	6.04	6.05	6.25	6.14	0.070	T=0.974
T2	6.04	6.43	5.87	6.12	6.09	6.11		P=0.958
T3	6.07	5.92	6.14	6.16	6.01	6.06		
T4	5.72	6.30	6.29	6.25	5.79	6.07		
Period mean	6.04	6.18	6.09	6.14	6.04			T×P=0.934
Albumin (g/dL)								
T1	2.65	2.82	2.76	2.84	2.78	2.77	0.022	T=0.461
T2	2.76	2.63	2.83	2.84	2.87	2.79		P=0.073
T3	2.70	2.86	3.05	2.71	2.76	2.82		
T4	2.70	2.82	2.94	2.55	2.58	2.72		
Period mean	2.70	2.78	2.89	2.73	2.75			T×P=0.209
Globulin (g/dL)								
T1	3.66	3.24	2.96	3.21	3.48	3.31	0.068	T=0.950
T2	3.28	3.80	3.04	3.28	3.22	3.33		P=0.661
T3	3.37	3.06	3.10	3.45	3.25	3.24		
T4	3.03	3.47	3.35	3.70	3.22	3.35		
Period mean	3.34	3.39	3.11	3.41	3.29			T×P=0.764
Blood urea nitrogen (mg/dL)								
T1	30.7	30.6	30.2	30.8	30.0	30.5	0.296	T=0.685
T2	30.8	29.6	30.8	29.9	30.2	30.3		P=0.759
T3	31.4	30.7	29.8	30.1	30.6	30.5		
T4	30.3	27.4	30.6	29.7	30.0	29.6		
Period mean	30.8	29.6	30.3	30.1	30.2			T×P=0.988

T1: no supplement; T2: 3% amla; T3: 3% fenugreek; T4: 1.5% amla + 1.5% fenugreek

Absence of effect on glucose despite varying dietary supplements infers an effective homeostatic mechanism in kids to maintain normal metabolic processes. The effect of plant additives on blood glucose is inconsistent in the literature. For instance, while quillaja saponins decreased blood glucose in Barbarine lambs (Nasri et al., 2011), medicinal herbs and fenugreek increased blood glucose in buffaloes (El-alamy et al., 2001). By contrast, Alamer and Basiouni (2005) reported a decrease in blood glucose with fenugreek supplementation in goats.

The concentration of triglycerides and cholesterol did not differ across treatments, and the interaction between treatment and period was non-significant. This broadly reflects the absence of any deleterious effect on fat metabolism with amla and fenugreek supplementation. Such a trend was also supported by previous research with fenugreek supplementation at a dietary level of 3% (Mir et al., 2013) and up to 20% (Al-Saiady et al., 2007) in goats, and 400 g of fresh amla fed to dairy cows (Tilahun et al., 2022).

There was no difference in plasma total protein, albumin, globulin and BUN among experimental goats (Table 2). Plasma protein level is dictated by level of dietary protein, while its level is altered in liver and kidney disease, but extremely low values are reported in inanition in animals (Kaneko et al., 2008). When tannin-rich leaf meals are fed, total protein did not vary (Anbarasu et al., 2004); however, saponin-containing fenugreek decreased total protein and albumin in goats (Mir et al., 2013). Furthermore, BUN declined with increasing condensed tannin (0, 1 and 2%) levels in the diet contributed through a mixture of dried tree foliages (Dubey et al., 2012). It is known that the BUN level is dictated by the extent of protein degradation (deamination) and subsequent capture of resultant ammonia by microbes in the rumen (Kaneko, 1997; Sharma et al., 2014). Indeed, the diets used in the present study did not vary in total protein levels, and it is probable that the plant bioactives tested did not modify protein metabolism to cause any variation in BUN.

CONCLUSION

It was concluded that supplementation of diets of growing goats with either amla or fenugreek at a maximum inclusion of 3% in diets had no influence on the haematological profile, reflecting a normal metabolism of energy, protein and fat. Hence, these prove to be safe natural feed additives for use in goat production.

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