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Effects of Formic and Propionic Acid as Silage Additive on Haematology and Biochemical attributes in Growing Cattle

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ABSTRACT

This study aimed to evaluate the effect of formic and propionic acid, alone or in combination as silage additive on haematological and biochemical attributes in growing Sahiwal cattle. A total of 24 growing Sahiwal heifers were randomly allocated into four groups (n=6) on body weight (117±5.0 kg) and age (10±2.0 months) basis. The experimental heifers either received a basal total mixed ration (TMR) having ensiled sorghum fodder without organic acids (S_C) or were fed on TMR having silage preserved with 0.5% formic acid ($S_{0.5\%FA}$), 0.5% propionic acid ($S_{0.5\%PA}$) or 0.25% formic acid and 0.25% propionic ($S_{0.25\%FA+0.25\%PA}$). Feeding of silage treated with formic and propionic acids did not exert any effect on dry matter intake (DMI) while average daily gain (ADG) and feed efficiency were better (P<0.05) in $S_{0.5\%FA}$ group. Treatments, days of feeding and their interaction did not affect red blood cells (RBCs) count, granulocyte count, lymphocytes count, mean corpuscular volume (MCV), mean cell haemoglobin (MCH) and mean corpuscular hemoglobin concentration (*MCHC*). However, white blood cells (WBCs) and platelet (PLT) count were significantly lower (P<0.05) in $S_{0.5\%FA}$ group and haemoglobin (Hb) concentration and packed cell volume (PVC) value in 0.5% $S_{0.5\%PA}$ group. No significant differences in the alanine transaminase (ALT), aspartate aminotransferase (AST) and creatinine were observed among four different groups, however; mean plasma alkaline phosphatase (ALP) concentration was higher in $S_{0.5\%PA}$ group. Treatments did not affect plasma Ca and P levels. In conclusion, feeding of silage preserved with formic and propionic acid, alone or in combination had no adverse effect on performance, haematological attributes and liver and kidney function test.

Key words: Biochemical, Cattle, Formic acid, Haematology, Propionic acid, Silage

INTRODUCTION

India is gifted with the large diversity and largest livestock population in the world with 299.6 million populations of cattle and buffaloes (DAHDF, 2012). Inspite of achieving the highest milk production, the performance of Indian cattle has been extremely poor and average productivity of livestock is lower compared to the world average. Poor performance of indigenous cattle is due to more populations of nondescript animals and deficiency of quality feed and fodders. While the livestock population is increasing, the gap between the requirement and availability of feed and fodder is increasing primarily due to decreasing area under fodder cultivation and reduced availability of crop residues as fodder. The country faces a net deficit of 35.6% green fodder, 10.9% dry crop residues and 44% concentrate feeds (IGFRI, 2013). This clearly indicates that as most of the livestock are underfed, they are not able to perform optimally. It is imperative to arrange sufficient good quality feed and fodder for efficient utilization of the genetic potential of the various livestock species and sustainable improvement in productivity.

The only way to meet the increasing fodder needs of livestock is to look for alternative options of fodder, silage being one of them. Silage is green succulent roughage preserved more or less in its original condition, with a minimum deterioration and minimum loss with respect of various nutritive constituents of fodders (Satter and Reis, 2012). Silage quality can be variable and the only way to effectively control the quality is by the use of additives. Silage additives are added to improve the fermentation process, reduce losses, improve aerobic stability and hygienic quality of the

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silage, limit secondary fermentation, and increase the nutritive value of the silage, resulting in increased animal production (Merensalmi and Virkki, 1991). Organic acids like formic and propionic acids as silage additives are effective in reducing yeast and molds which are responsible for the aerobic deterioration of silages. The use of silage with a high content of fermentation acids may result in a substantially lower energy yield for rumen microorganisms than the use of silage of restricted fermentation (Chamberlain, 1987). In terms of animal performance, feed intake and live weight gain were higher with formic acid-treated silage than untreated or inoculant-treated silages (Haigh *et al.*, 1987). Forage ensiled with formic acid has produced weight gains or milk production equal to or greater than wilted untreated silage (Waldo and Derbyshire, 1971). Forage ensiled with propionic acid brings about an increase in silage intake, which leads to increased live weight gain (Nadeau and Arnesson, 2016). The objective of this study was to examine the relative merits of formic and propionic acid, alone or in combination as silage additives on haematological attributes and liver and kidney function tests in growing Sahiwal cattle.

MATERIALS AND METHODS

Animal care procedures were approved (approval number, 121/IAEC/18) and conducted under the established standard of the Institutional Animal Ethics Committee (IAEC), constituted as per the article number 13 of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) rules laid down by the Government of India.

In the present study, the green sorghum fodder (*Sorghum bicolor*) with moisture content of 65-72% was ensiled either without organic acid (S_c) or with 0.5% formic acid ($S_{0.5\%FA}$) or 0.5% propionic acid ($S_{0.5\%PA}$) or combination of each formic and propionic acid at 0.25 % each ($S_{0.25\%FA+0.25\%PA}$). The whole fodder was chopped into 2-3 cm pieces by using tractor operated forage chopper (Ensiladeria JF40Max, NB Maquinas Ltd., Brazil). After treatment, chopped fodder was packed in polythene bags (1.5 meters in height and 0.5 meter width) having capacity of 50 kg. After 90 days of ensiling, prepared silage was fed to the experimental animals of respective group.

A total of 24 growing Sahiwal heifers were selected from the cattle herd maintained at LFC, DUVASU, Mathura. Experimental animals were housed in a well-ventilated shed having the proper arrangement for individual feeding and watering without having access to the other animal's diet. Deworming of all the experimental animals were done before the start of the experiment by oral administration of Fentas bolus (Intas Pharmaceuticals Pvt. Ltd., India) at the dose level of 10 mg/kg body weight. Animals were let loose every fortnightly for exercise. The nutrient requirements of experimental heifers were met by the feeding of total mixed ration (TMR) consisted of concentrate: silage: wheat straw in the proportion of 40:40:20 following NRC (2001) guidelines. Selected heifers were randomly allocated into four groups (n=6) on body weight (117±5.0 kg) and age (10±2.0 months) basis. The experimental heifers either received a basal TMR having silage without additives (S_C) or were fed on TMR containing 0.5% formic acid ($S_{0.5\%FA}$), 0.5% propionic acid ($S_{0.5\%PA}$) and 0.25% formic acid and 0.25% propionic ($S_{0.25\%FA+0.25\%PA}$) added silages. Ingredients and nutrients composition of TMR fed in different groups are presented in Table 1. TMR was prepared daily by hand mixing and offered at 0900 h in all tests. The calves were fed the TMR in such an amount that at least 5% refusals were left daily per animals. Fresh drinking water was offered *ad libitum* twice daily at 0800 h and 1700 h.

The experimental calves were monitored daily for DMI and fortnightly for growth performance and feed efficiency measures. To increase the accuracy of the estimation of ADG, calves were weighed at fortnight intervals in the early morning (0600 h) before offering feed and water. Feed conversion ratio (FCR) and feed conversion efficiency (FCE) were observed as feed efficiency measures. Feed-to-gain ratio or FCR was calculated by the amount of DMI (kg) required for unit (per kg) weight gain by animals during the trial period. FCE was calculated as the ratio between ADG (kg) and DMI (kg) by animals during the trial period. The representative samples of TMR offered and residue left were dried in a hot air oven at 60ºC till a constant weight was attained and ground in a Wiley mill to pass a 1-mm sieve. Processed samples were pooled animal wise and stored at the dry place for the further analysis of DM (Method 973.18c), CP (Method 4.2.08), EE (Method 920.85), and total ash (Method 923.03) as per methodology of AOAC (2005). aNDFom, aADFom and ADL were determined according to the procedures described by Van Soest *et al.* (1991).

Peripheral blood samples were collected in heparinized vacutainer tubes (BD Franklin, USA) by venipuncture of anterior vena cava at 0, 30, 60 and 90 days post-treatment for the analysis of haematological and plasma attributes. A fraction of whole blood was used for analysis of RBCs count, WBCs count, PLT count, granulocytes count, lymphocytes count, Hb concentration, PVC or HIT value, MCV, MCH and *MCHC* by using automatic analyzer (Celltac alpha CM, Nihon, Kohden, Pvt. Ltd, Surat, India). Remaining amount of blood samples were centrifuged at 3000 rpm for 30 min to separate the plasma from packed erythrocytes. Plasma samples were stored at -20ºC until further analysis of ALT, AST, ALP, creatinine, Ca and P levels by using the automated biochemical analyzer

Table 1. Ingredients and nutrients composition of TMR fed during feeding trial (g/kg DM basis or as mentioned)

Attributes	Treatments$^{\alpha}$			
	S_C	$S_{0.5\%FA}$	$S_{0.5\%PA}$	$S_{0.25\%FA+0.25\%PA}$
Ingredients composition				
Sorghum silage	400	400	400	400
Wheat straw	200	200	200	200
Mustard oil cake (solvent extract)	128	128	128	128
Ground barley grain	104	104	104	104
Gram husk	80	80	80	80
Wheat bran	80	80	80	80
Micronutrient mixture$^{\beta}$	8	8	8	8
Chemical composition				
DM 652.9	654.1	653.3	654.5	
CP 126.8	126.2	127.1	126.8	
EE 31.7	31.4	32.1	32.6	
Ash 87.0	87.4	88.8	88.6	
aNDFom	560.4	553.6	561.2	556.0
aADFom	317.2	314.0	318.4	320.0
ADL 62.1	64.4	62.2	62.1	
Total CHO	754.5	754.9	752.1	752.0
NFC 194.1	201.3	190.9	196.0	
Ca 7.2	7.1	7.3	7.2	
P 3.2	3.2	3.3	3.2	
ME, MJ/kg DM	8.28	8.24	8.20	8.24

$^{\alpha}$Sc, silage without organic acid; $S_{0.5\%\,FA}$, silage with 0.5% formic acid; $S_{0.5\%\,PA}$, silage with 0.5% propionic acid; $S_{0.25\%FA+0.25\%PA}$, silage with 0.25% of formic and propionic acid; $^{\beta}$Micronutrient mixture consisted (kg^{-1}) of 700,000 IU of vitamin A, 70,000 IU of vitamin D_3, 250 mg of vitamin E, 190 g of Ca, 90 g of P, 50 g of Na, 19 g of Mg, 1.2 g of Cu, 9.6 g of Zn, 1.5 g of Fe, 6.0 g of Mn, 325 mg of I, 150 mg of Co, 10 mg of Se.

(BS-120 Chemistry Analyzer, Shenzhen Mindray Biochemical Electronics Co. Ltd.).

Data of the study were subjected to analysis of variance using the General Linear Model (GLM) procedure of the Statistical Software Package (SPSS for windows, V21.0; Inc., Chicago, IL, USA). The effect of formic and propionic acid as silage additive on performance, haematology and plasma attributes were analyzed by using the following model:

$$Yijk=\mu + Ti + Dj + (T\times D)ij + eijk$$

Where; *Yijk* is dependent variable, ì is overall mean of the population, *Ti* is mean effect of the treatment, *Dj* is mean effect of days of sampling (j=0, 30, 60 and 90 days of dietary treatment), *(T×D)ij* is effect of the interaction between treatment and period and *eijk* is unexplained residual element assumed to be independent and normally distributed. Individual animals were used as the experimental unit for all data. The statistical difference between the means was determined by using "Tukey's honest significant difference (HSD) test". Significance was determined at P<0.05 and the values are presented in the tables.

RESULTS AND DISCUSSION

Daily DMI, ADG and feed efficiency in $S_{C,}$ $S_{0.5\%FA,}$ $S_{0.5\%PA}$ and $S_{0.25\%FA+0.25\%PA}$ groups are depicted in Table 2. Treatments did not affect DMI while ADG was better (P<0.05) in $S_{0.5\%FA}$ group. FCR showed significant (P<0.05) effect of treatment and found better in heifers of $S_{0.5\%FA}$ group than that of other groups. Although the FCE was observed numerically higher in $S_{0.5\%FA}$ group but overall mean values showed non-significant effect in four different groups.

The main factors affecting silage intake were palatability, forage digestibility, rumen degradability and the concentrations of nitrogen and fibre fractions (Steen *et al.,* 1998). The intake response to formic acid ensiled fodder observed in the current experiment has also been reported previously with dairy cows (Waldo *et al.,* 1971). In contrary to the findings of the present study, research reviewed by Thomas and Thomas (1988) shows that addition of formic acid to grass silage increased voluntary intake by 9%. A study with sheep indicated a 13% increase in intake of grass silage treated with formic acid (Rooke *et al.,* 1988). No significant differences occurred, but all data favoured formic acid treated silage over untreated silage. Formic acid appeared to reduce the concentration of acetic acid and ammonia, which are fermentation products that have been implicated in the regulation of silage intake (Gill *et al.,* 1988; Thomas and Thomas, 1988). Additionally, formic acid restricts fermentation and protein degradation during ensiling, which can have a positive effect on animal performance (Winters *et al.,* 2001).

Results suggest that sorghum silage treated with preservatives has the potential to replace fodder in diets fed to growing cattle. Woolford (1984) reported only high percentages of unbuffered propionic acid (1.0 to 3.0% of the DM) were deemed to be effective inhibitors of aerobic deterioration of silage. Such high application rates often improved DMI and growth (Mann and McDonald, 1976). Results of a study carried out by Nadeau *et al.* (2000) revealed that formic acid added silage resulted in higher feed intake and improved growth performance. The amounts of the additives (0.25 to

Table 2. Effect of silage preservatives on the performance

Attributes	Treatments				SEM	P-value$^{\alpha}$		
	Sc	$S_{0.5\%FA}$	$S_{0.5\%PA}$	$S_{0.25\%FA+0.25\%PA}$		T	D	T×D
DMI, kg/d	4.76	4.90	4.63	4.75	0.25	0.619	0.042	0.798
ADG, kg/d	0.514^{b}	0.591^{c}	0.463^{a}	0.504^{ab}	0.03	<0.001	0.398	0. 046
FCR	8.92^{ab}	8.59^{a}	9.62^{b}	9.45^{b}	0.24	0.046	0.578	0.882
FCE	0.120	0.127	0.110	0.112	0.01	0.556	0.429	0.994

$^{a-c}$Mean values within a row with unlike superscript letters were significantly different for each dietary treatment (P<0.05); $^{\alpha}$T represents the main effect of treatment; D represents the main effect of days of feeding trial; T×D represents the interaction between the main effects of treatment and days.

0.5%) that we used in our study were considerably less than those used in previous studies. In the present study, significantly improve FCR was found in $S_{0.5\%FA}$ group as compared to other groups which is in accordance to the findings of O'Kiely and Moloney (1994). Winters *et al.* (2001) reported 26% improvement in feed efficiency in steers fed direct-cut formic acid-treated grass silage (3.3 L/ton).

The RBCs count, granulocyte count, lymphocytes count, MCV, MCH and MCHC showed non-significant effect of treatments (Table 3). Whereas, WBCs and PLT count were significantly lower (P<0.05) in 0 $S_{0.5\%FA}$ group and Hb concentration and PCV values were lower (P<0.05) in $S_{0.5\%PA}$ group. The RBCs (7.98-8.65×10^6/µl blood), WBCs (11.10-14.91×10^3/µl blood), PLT count (252.38-333.00×10^3/µl blood), Hb (8.96-10.05 g/100 blood) and PCV (29.18-32.65%) were in the range as reported by Singh *et al.* (2019). No valid justification available regarding the role of organic acid treated silage on haematological attributes. Variation in WBCs, PLT, Hb, PCV or HIT, and MCHC could be due to biological factors. MCHC value varies significantly in the last month of study however; the slight change in MCHC may also result from the hemolysis of the analyzed material (Brucka-Jastrzebska *et al.,* 2007). The Hb content and PCV of blood in cattle significantly (P<0.05) increased with the use of organic acid in water during the thermal stress conditions (Ali *et al.,* 2013). While in birds, organic acid supplementation increased RBC count, WBC count and Hb content (Vinus *et al.,* 2017). Abdel Raheem (2016) revealed that there were no significant differences between different experimental groups in Hb concentration, WBCs, RBCs count, PCV, MCV, MCH and MCHC and lymphocyte percentage on feeding of organic acid to broiler Chickens. Al-Saad *et al.* (2014) showed that

Table 3. Effect of silage preservatives on haematological and biochemical parameters

Attributes	Treatments				SEM	P-value$^{\alpha}$		
	Sc	$S_{0.5\%FA}$	$S_{0.5\%PA}$	$S_{0.25\%FA+0.25\%PA}$		T	D	T×D
Haematological attributes								
RBC count, 10^6/µl	8.65	7.98	8.36	8.65	0.16	0.069	0.392	0.629
WBC count, 10^3/µl	14.03^{ab}	11.10^{a}	14.91^{b}	13.03^{b}	0.82	<0.001	0.611	0.429
PLT count, 10^3/µl	322.75^{ab}	252.38^{a}	333.00^{b}	315.58^{ab}	18.20	0.023	0.449	0.721
Granulocytes count, %	29.83	30.20	33.51	29.70	0.91	0.742	0.493	1.000
Lymphocytes count, %	63.90	63.48	63.23	65.70	0.56	0.879	0.583	0.998
Hb level, g/100 ml	9.38^{ab}	9.21^{ab}	8.96^{a}	10.05^{b}	0.23	0.011	0.429	0.671
PCV, %	31.26^{ab}	30.34^{ab}	29.18^{a}	32.65^{b}	0.54	0.018	0.177	0.442
MCV, fl	36.36	38.30	35.24	38.57	0.80	0.075	0.539	1.000
MCH, pg	10.93	11.63	10.84	11.85	0.25	0.086	0.662	0.791
MCHC, g/100 ml	30.28	30.38	30.72	30.70	0.11	0.083	0.331	0.522
Biochemical attributes								
ALT, IU/L	6.37	6.17	6.60	6.32	0.19	0.083	0.429	0.728
AST, IU/L	26.41	25.59	27.03	27.10	1.35	0.381	0.118	0.332
ALP, IU/L	27.63^{a}	29.28^{a}	32.15^{b}	28.04^{a}	1.02	<0.001	0.093	0.591
Creatinine, mg/100 ml	1.19	1.17	1.18	1.21	0.01	0.400	0.483	1.000
Ca, mg/100 ml	9.70	9.71	10.10	10.14	0.62	0.091	0.392	0.889
P, mg/100 ml	6.46	6.33	6.21	5.88	0.18	0.401	0.755	1.000

[a-c]Mean values within a row with unlike superscript letters were significantly different for each dietary treatment (P<0.05); [α]T represents the main effect of treatment; D represents the main effect of days of feeding trial; T×D represents the interaction between main effects of treatment and days.

feeding of propionic acid in diets resulted in an increase in WBC concentration while there was no effect on Hb and PCV values. Vinus *et al.* (2017) found that propionate feeding increases RBC, WBC and Hb concentrations significantly (P<0.05) than untreated groups. Supplementation of salts of organic acids reduced the number of heterophils and improved the lymphocytes count in different treatments as compared to the control group. Abdel-Hamid and Omar (2019) suggested that there was an increased concentrations of RBC, WBC and Hb when diet treated with various level of formic acid as compared to the control group in rabbits. EL Naggar *et al.* (2017) investigated the effect of formic and citric acid and concluded that increased RBCs and Hb as compared to control.

Studied biomarkers of liver and kidney function tests in present study were ALT, AST, ALP and plasma creatinine levels (Table 3). No significant differences in the ALT, AST and creatinine were observed among four different groups. However, mean plasma ALP concentration was higher in $S_{0.5\%PA}$ group. In this study, biomarkers of liver and kidney function tests have been used to monitor and evaluate health and nutritional effect of silage preservatives in growing cattle. The information regarding the effect of feeding of organic acid added silage on biochemical parameters is lacking. Plasma parameters values measured were within the normal range for healthy dairy animals as recorded by George *et al.* (2010); Wood and Quiroz-Rocha (2010). Although plasma AST level in last month of study shows significant results but value was within the reference range (Radostits *et al.,* 2000). Plasma ALP was found significant during the whole duration of study but value were within range (18-153 IU/L) and does not exert adverse effect (Latimer *et al.,* 2003). Different levels of organic acid showed a non-significant effect on serum GOT, GPT, uric acid and creatinine concentrations as reported by El-Shenway and Ali (2016) and Abdel-Fattah *et al.* (2008). Finding of Ragaa *et al.* (2016) also showed that formic acid supplementation did not affect AST, ALT and uric acid levels. Naggar *et al.* (2017) observed that supplementation of formic and citric acid decreased serum AST and ALT levels compared to control group. Creatinine, the waste product produced via the catabolism of phospho-creatine is a basic parameter reflecting kidney function (Stojevic *et al.,* 2005). In the present study; plasma creatinine level observed was 1.17-1.21 mg/100 ml that fell within the physiological range as observed by Radostits *et al.* (2008) and Smith (2009). In a study conducted by Naggar *et al.* (2017) it was reported that supplementation of formic and citric acid decreased serum creatinine levels as compared to unsupplemented group.

The results show that addition of silage preservatives does not alter the plasma concentration of Ca and P. Observed mean values of Ca were within the range of 9.70-10.14 mg/100 ml. Similarly, serum concentration of P ranged from 5.88-6.46 mg/100 ml, which was within physiological range as reported by Latimer *et al.* (2003). Similar to the findings of the present study, Vinus *et al.* (2017) reported similar serum Ca level in organic acid as well as unsupplemented group while serum P level was improved in organic acid-treated groups. In contrast to the findings of the present study, Jongbloed *et al.* (2000) and Hernandez *et al.* (2006) observed a positive interaction among organic acids and plasma Ca and P levels in growing pigs. Abdel-Fattah *et al.* (2008) also found higher plasma Ca and P in organic acid group than those given unsupplemented diet. They concluded that the increase in Ca and P level in plasma produced by addition of organic acid may be attributed to lowering of GI tract pH by using these acids, which increases the absorption of such minerals from gut into blood.

CONCLUSIONS

The findings of this study revealed that feeding of silage preserved with 0.5% formic acid in heifers improved growth performance and feed conversion while feed intake remains similar. Feeding of formic and propionic acid, alone or in combination as silage additive did not exert any adverse effect on haematological and biochemical attributes as values ranged within normal physiological range.

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