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Effect of supplementation of molasses (*Saccharum officinarum*) on growth performance and cortisol profile of growing pig in north eastern hill ecosystem of India

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Abstract
This study was performed to investigate the effect of dietary supplement of molasses as summer feed on growth performance and blood cortisol profile of growing pigs to ameliorate the adverse effect of heat stress. A total of twenty-four, (75% Hampshire × 25% Niang Megha) crossbred grower pigs (3.5 months old; average body weight : 18.57±0.67 kg) were randomly divided into two homogenous groups viz., T1 (Control) : CP:17.17% and ME:3320.6 kcal/kg and T2 as basal diet with 11% Molasses ; CP: 15.31% ; ME: 3376 Kcal/kg; n=12 pigs per treatment in a completely randomized design for 90 days experimental periods. All the two groups diet were isocaloric in nature and fulfilled requirement as per NRC (2012). The results supplemented treatments had significant differences in average dry matter intake (1416±2.03 vs 1386±2.12 g/d), body weight gain (37.70±0.16 vs 35.24±0.06kg) and average daily weight gain (418±4.03 vs 391±4.08g/d) as compared to control. Better ($P<0.001$) feed conversion ratio (3.38 vs 3.54) was observed in the supplemented treatments and also significant ($P<0.01$) reduce serum cortisol (3.33vs 3.60 µg/dL) than the control group. It could be concluded that formulated summer feeds affect growth performance but also improving the feed intake and lowered the blood cortisol profile in treated growing pigs during summer. It could be best option as energy sources have to be developed based on nutritive value and anti-stress property of the existing feed resources and increasing pig production under sub-tropical region of north eastern India.

Keywords: Molasses, heat stress, cortisol, performance, North eastern hill

Introduction

Pigs are preferred as valued livestock for rearing especially in marginal and small holding farming system due to its unique potential scope in socio economic livelihood of tribal farmers especially in north eastern region of the country. Feed accounts for 65- 75% of the total cost in the world pig production [1], considerable research efforts have been spent to reduce its cost by using cheap locally available feed resources. It becomes, therefore, strategically important to find new sources of energy for animal feeding, in order to save cereal grains for human consumption. Cane molasses is an alternative cheap source of energy [2] but it is rich in niacin and pantothenic acid [3] and minerals [4] as compared to other energy rich feeds, cereal grains. Molasses has been used as an animal feed for livestock and poultry stock in tropical regions [5] [6, 7]. Commercially it is included in animal rations at a level of 15% for cattle and pigs, 8% for calves and sheep, and 5% for poultry [8].It is used with dry feeds of animals to increase palatability, setting dust, carrier for other essential nutrients, serving as a binder for pelleting. Furthermore, it acts as an excellent source of trace minerals and some micro-elements [9] [10] [11]. The use of molasses in animal feeding is limited, because overconsumption of molasses causes molasses toxicity in ruminants and laxative effects in monogastric animals. Other problems that limited the use of high levels of molasses are the difficulty of mixing quantities in excess of 20% of the diet. Thermal stress is major issue and severely affected on growth performance of grower pigs under sub-tropical and tropical climates [12]. It continues to threaten global food security and meat production especially in developing countries [13]. High frequency of extreme weather events, increase in global average surface temperature of 0.8 to 2.6 °C by 2050 and 1.4 to 5.8 °C over the next century [14] predicted the negative effects on livestock production [15] in most animal producing areas [16].

Dramatically, impact of heat stress effect indirectly on reduced production performance and increase health care cost which ultimately results in huge exacerbated economic losses [17]. Nutrient requirements are altered during heat stress which results in a need for reformulation of rations [18]. Proper feeding strategies planning and ideal nutritional requirements need to change based on environmental conditions or climate change are still to be elucidated which can only be achieved by understanding the impacts of thermal stress that specific physiological response, nutrition and metabolism. Nutritional modification through mitigation may be helpful to reduce heat stress and improved growth rate. There is still paucity information available on assessment of nutritional management for pigs during heat stress in these regions. Therefore, it is urgent need of nutritional intervention for development of feed formulation for improving productivity and alleviation of heat stressed pigs. The aim of this study was to evaluate the potential of molasses as dietary summer feed on growth performance and cortisol profile in growing pigs.

Materials and Methods

Study Area

The study was conducted at the Livestock farm, Division of Livestock Production, ICAR Research Complex for North Eastern Hill Region, Umiam, Meghalaya, India, located at 24° 58‟ N to 26° 07‟N latitudes and 89° 48‟E to 92° 51‟ E longitudes with an altitude of 1010 meter above mean sea level. The Annual rainfall varies from 2239 mm to 2953 mm. The annual maximum and minimum temperatures range from 21.1 °C to 29.2 °C and from 7.0 °C to 20.9 °C, respectively. The annual maximum and minimum temperature humidity index (THI) range from 67.82 to 85.02 with an average of 75.95. The average relative humidity of the region ranges from 71.1 to 88.3% in the morning to 41.7 to 73.7% in the afternoon.

Animals, experimental design and management

A total of twenty four, (75% Hampshire × 25% Niang Megha) crossbred growing pigs at 3.5 months with an average initial body weight of 18.57±0.67 kg were randomly divided into two homogenous groups of twelve pigs in each group on the basis of comparable age and uniform body weight in a completely randomized design. The study was conducted from 25th May to 25th August, 2019 with a daily minimum and maximum temperature averaging 16.3 and 29.7 °C. The experimental diets were formulated from the local available feed ingredients (molasses) in a way to be isocaloric to meet nutrients requirements [19] of growing pigs are shown in Table 1. Adjustment period (acclimatization) of 10 days was carried out to habituate the animals with experimental feed. All pigs were dewormed before starting the experiment and maintained in a hygienic condition with uniform managerial practices throughout the study period. Pigs were fed equal meals twice daily at 0900 and 1500 hours. Daily feeding rate was 4% of the pigs‟ live weight [20]. Water was given to the pigs ad libitum throughout the period of the standardization trial. The feeding trial was lasted for ninety (90) days.

Assessment of feed intake, body weight gain and feed conversion ratio

Pigs were weighed at the start of the experiment and subsequently on weekly basis. The amounts of feed offered and refused were recorded daily and their difference is considered as daily feed intake. Weight gained was determined by obtaining the difference between initial weight and final weight of each group. Feed conversion ratio was calculated as feed per gain.

Blood profile sampling and analysis

Pigs were handled very carefully to avoid a circadian variation of cortisol during summer. Collection of blood samples was done in the early morning between 8:00-10:00 am at 30 days interval during 90 day experimental period. The experimental animals were restrained in ventro- dorsal position and 5-10 ml blood were collected directly from anterior vena cava under aseptic condition by using 15 gauge 4 inches needle using a disposable vacutainer tube without anti- coagulant (Becton Dickinson, Franklin, USA) from all the animals. After centrifugation of 3000 rpm for 15 minutes to obtained serum and were stored at -20oC for further analysis. For analysis of serum cortisol levels were determined using micro plate enzyme immunoassay as per standard protocol Cortisol EIA assay kit (Item no: 5000360, Cayman chemical, USA).

Statistical analysis

Data from feed intake, body weight change, feed conversion ratio and cortisol parameters were subjected to analysis of variance (ANOVA) using SPSS statistical software [21]. Variability in the data was expressed as the pooled standard error and at $P<0.05$ was considered to be statistically significant as per statistical procedures [22].

Results and Discussion

Effect of diets on growth performance and cortisol level

Results of the growth performance of pigs used in this study are presented in Table 2. The average daily DM intake was significantly higher ($P<0.001$) in treatment group than the control group. Incorporation of molasses in growing pigs diets resulted in improved performance of the experimental group with regard to all the productive parameters.

Table 1: Ingredient composition (%, as-fed basis) of control and experimental diets for grower pigs to alleviate heat stress

Ingredient (%)	Experimental diets	
	Control	**Summer-feed**
Maize grain	52	47
Molasses	-	11
Mustard oil	-	-
Wheat bran	23	20
Soybean meal	10.5	9.5
Groundnut cake	12	10
Mineral Mixture[1]	2	2
Salt	0.5	0.5
Calculated nutrient composition		
CP %	17.17	15.31
ME (Kcal/Kg)	3320.6	3376.6

Table 2: Effect of summer feed on growth performance and stress biomarker of growers pigs Parameters

Age (months)		**3.5 to 6.5**	
Body weight (kg)	**Control (n=12)**	**Treatment (n=12)**	**SL**
Initial BW, kg	18.38±0.78	18.34±0.56	ns
Final BW, kg	53.62 [a]±0.84	56.13 [b]±0.72	*
Weight gain (kg)	35.24 [a]±0.06	37.70 [b]±0.16	*
ADMI (g/d)	1386[a]±2.12	1416 [b]±2.03	**
ADG (g/d) FCR	**391[a]±4.08 3.54[a]**	**418[b]±4.03 3.38[b]**	*
Cortisol(µg/dL)			
1. 30 days	3.85	3.63	**
2. 60 days	3.56	3.21	**
3. 90 days	3.38	3.16	**
Overall	3.60[a]	3.33[b]	**

*a, b Means within the same row not bearing a common superscript letters differ significantly at ** = P<0.001; ns = not significant at (P>0.05); ADG = average daily body weight gain; BW = body weight; ADMI =Average dry matter intake; FCR: feed conversion ratio; SE = standard error; SL = significant level

There is significant difference (P<0.005) among the diets which was mostly in growth phase. This could be attributed to the differences in the chemical composition among the two diets as summarized in Table 1. The average body weight gain (37.70±0.16 vs 35.24±0.06 kg), ADG (418 vs 391 g/d) and FCR (3.38 vs 3.54) was significantly (P<0.005) affected by dietary treatments with a higher value than the control group. However, the overall serum cortisol concentration (3.33 vs 3.60 µg/dL) was significantly lower (P<0.001) in summer feed as compared to the control group during heat stress. In comparison with summer feed, pigs fed with control diet exhibited more heat stress with significant reduction in growth rate. Firstly, this might be attributed to reduced growth performance can be related to high fiber and its consequences in the energy or protein digestibility coefficient. In fact, presence of dietary fiber in diets is known to greater post-prandial thermogenic response or emits metabolically heat increment [12, 23] in the present study.

In our experiment, grower pigs fed with formulated summer diet of highly digestible energy (11% molasses) with low protein (15.31%) with little supplement of vitamins (C, E), mineral (Se) and electrolytes (Potassium chloride/Sodium bicarbonate) improved growth rate and reduce heat stress in treated pigs. There was significant effect on body weight and average daily gain in the current study. Similar finding was also supported by Suresh Kumar *et al.* [24] and Ly *et al.* [25]. Conversely, the present findings are contrary to the results reported by Feoli *et al.* [26] and Xande *et al.* [27]. However, this study showed that by reducing dietary protein (15.31%) in summer feed provides less metabolic heat generate in pigs. The present findings are in close agreement with the results reported by Noblet (2019) [28] and Lopez *et al.* [29]. Further, a benefit of molasses inclusion in summer diet is increased energy concentration of feed in order to compensate for decrease feed intake. Similar results were obtained by Brooks and Iwanga (1956) [30] and Perez (1995) [31]. Molasses also provides essential vitamins [32] and minerals [33] to commensurate with the high energy feed in grower pigs diet to reduce heat stress. Besides, supplementation of vitamin E and Se also produce combined or supra-nutritional effect for improving feed intake and oxidative balance for alleviating the physiological response to heat stressed pigs (Liu *et al.* [34]). Level of serum cortisol in treatment is slightly lower than control group but within the normal range (Radostitis *et al.* [35]) due to low protein with molasses. Whereas, high concentrate feed in control diet fed to control group resulting into refusal feed intake and compromising growth rate during heat stress has been found to increase blood cortisol levels. This reduce feed intake may be contributed to increase serum cortisol as a compensatory mechanism to low energy intake as a regulator of metabolizable energy in the body. Serum cortisol level increases hepatic gluconeogenesis and the peripheral release of substrates (muscles) required for gluconeogenesis were obtained by Velazco *et al.* [36] and Maduka *et al.* [37].

Conclusion
The result of the study inferred that supplementation with 11% molasses in feed exhibits ameliorative effects of heat stress in growing pigs. Utilization of locally available energy feed resources (molasses) could be used to increase nutrient density with little supplement of vitamins, mineral /electrolytes to reduce heat stress and improved pig production. Besides, not only increasing energy intake but also balanced dietary protein during hot stress in this region. However, this finding may be useful for improving growth performance and minimizing the adverse effect of thermal stress in pigs.

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