

# Growth Performance, Hematological and Mineral Profile of Post-Weaning Calves as Influenced by Inclusion of Pelleted-Concentrate Supplement Containing Essential Oils and Probiotics

**Research Article** 

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#### ABSTRACT

Two experimental factors (dietary treatment with or without pelleted-concentrate supplement (PCS) and sex) were investigated in crossbreed post-weaning calves to monitor their effects on performance, hematological profile, and plasma mineral concentration. Twenty-four post-weaning calves were classified according to sexually (either male or female) and randomly distributed into two dietary treatments that were included the first group controlled diet without PCS inclusion (CON) and second was CON plus 20% PCS inclusion (PCS). Results showed that calves received PCS resulted in higher daily gain and feed efficiency thereby had higher final weight in comparison with CON (P<0.01). These results can be associated with higher energy intake in the PCS group (P<0.05) although the dry matter intakes (DMI) were similar (P>0.05). Interaction effects were not detected on calves performance, hematology, and plasma mineral concentration (P>0.05). Hematological profile and plasma mineral concentration were not affected by dietary treatment, sex, and their interaction (P>0.05) except for hemoglobin in the PCS group were higher than the CON group (P<0.05). To conclude, the mixture of probiotics, essential oils and mineral premix was given in 20% PCS inclusion showed a synergistic beneficial effect as shown in an improved of calves performance (i.e daily gain, feed efficiency, and final body weight) without affecting blood composition profile as well as plasma calcium and phosphorus concentrations.

KEY WORDS calcium-phosphorus, concentrate supplement, crossbreed calves, feed efficiency, gain, hematological profile.

#### INTRODUCTION

Offering a high concentrate diet to beef cattle becomes a common practice in the feedlot industry. Primarily in postweaning calves, optimum growth is more achievable when essential nutrients and supplements are provided. Since the ban of antibiotics administration as a growth, the promoter has been becoming a great concern worldwide and public awareness has considerably increased regarding the meat quality (Salazar *et al.* 2019), the inclusion of natural additives to ruminant diets arises among beef cattle industry. In addition, post-weaning calves reared intensively needs not only high-quality concentrate but also adequate essential mineral and some beneficial additives. Providing these compounds is crucial to achieving optimal body development. The amount of highly digestible material and additional supplements are influencing livestock performance during the post-weaning period.

Body development in the post-weaning period also determines the quantity and quality of meat in the finishing period (Bhatti *et al.* 2012).

Among many types of additives that have been intensively studied, probiotics and essential oil (EO) are two of the most popular, especially because of high efficacy and commercially available. There is large evidence that probiotics and essential oils can be antibiotics alternatives due to their antimicrobial activity and showed a positive effect in improving post-weaning calf performance. It has been reported that probiotics and essential oils are effective to increase feed intake and efficiency of nutrients utilization and stimulating health by activating immune cells thereby promoting health performance and average daily gain (ADG) (Benchaar and Greathead, 2011; Froehlich et al. 2017). Probiotics, by many studies, exhibit a positive effect on most animal species although the efficacy is varied (Salazar et al. 2019). For instance, it improved post-weaned calves' weight gain and feed efficiency (Kelsey and Colpoys, 2018), immune function and performance of broiler chickens (Jamshidparvar et al. 2017), and egg production and eggshell quality of Japanese quails (Ayasan et al. 2006).

In regard to essential oil, it is has been widely used as a new class of feed additive to improve the intestinal microbiota of a variety of animals and have positive effects on calves' performance and healthy (Froehlich et al. 2017). Essential oils are chemically a mixture of some active  $(C^{10})$ including monoterpenes compounds and sesquiterpenes (C<sup>15</sup>), act as antimicrobial and antioxidant agents (Jayasena and Jo, 2013). However, the results of the EO application also vary depending on their sources, chemical composition, the doses used, and the synergistic effects among chemical compounds in the oils (Benchaar et al. 2013). The interaction between additives and diets are diverse and primarily important for the efficiency of beef cattle production (Salazar et al. 2019).

Although a large number of experiments have been addressed to answer the efficacy of individual additive or some mixtures specifically, there is limited research when the mixtures of additives are blended with a mineral supplement in a concentrate diet. Considering the above circumstances, in this present experiment, concentrate diet contained highly by-pass protein has been mixed with multi-supplement including probiotics, essential oils, mineral supplements and was labeled with pelleted concentrate supplement (PCS). The inclusion of such a commercial feed supplement is expected to have a synergistic beneficial effect on post-weaning crossbreedcalves health and performance. In addition, it is easier to be used directly by farmers in Indonesia. Therefore, this study was conducted to examine the effect of PCS inclusion on blood parameters, plasma mineral status, and average daily gain of post-weaning calves.

# MATERIALS AND METHODS

## **Experimental animal**

The experiment was carried out at the experimental farm in the Department of Animal Nutrition and Feed Science, Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, Indonesia from August to November 2017. Twenty four (24) Brahman crossbreed post-weaning calves, consisting of 12 males and 12 females with average initial body weight (BW) of  $101 \pm 24$  kg were used in this study. Calves were intensively reared in individual pens facilitated with individual feeding and bucket drinking water. The calves were adapted and habituated to 14 days in which during this time the calves received 3 kg of basal concentrate diet and forage given as total mixed ration (TMR) (dry matter basis) and ad libitum drinking water. The manure was removed daily to keep the pens visibly clean and dry. During the experimental period, calves were weighted bi-weekly to record weight gain.

## Feeding and experimental diets

Twenty four post-weaning calves were randomly distributed into four groups (2×2 factorial arrangement) following cross over design. Briefly, the post-weaning calves were separated according to sex (male and female). Two groups of calves in each sex were randomly allocated into two dietary treatments (n=6); the first group controlled diet without PCS inclusion (CON) and second was CON plus 20% PCS inclusion (PCS). Control diet was formulated by mixing 86% of concentrate and 14% forage while PCS treatment group was 66% concentrate + 20% PCS + 14% forage (DM basis). Both diets were offered as TMR to the calves. The concentrate consisted of wheat, pollard, cassava chip, rice bran, soybean meal, and molasses. In addition, the PCS was composed of corn grain, corn gluten meal (CGM), pollard, soybean meal (SBM), wheat flour, and AGROMIX Booster® (commercial product). The AGROMIX Booster® is composed of mineral mix Ca 26.45%, P 0.62%, K 0.22%, Mg 0.26%, Na 4.70%, S 681.7 ppm, Fe 1.44%, Zn 300.2 ppm, Cu 229.7 ppm, Se 0.541 ppm dan Co 7.7 ppm, vitamin A, D, E dan K, probiotics (Lactobacillus acidophilus, Enterococcus faecium, Saccharomyces cerevisiae) and blended essential oils processed from walnuts, olives, soybeans, lavender, eucalyptus, coconut, orange, peppermint and sesame seeds. The PCS was manufactured by PT. Widodo Makmur Perkasa at the feed mill plant in Bogor, West Java, Indonesia in the form of pellet (1.5 cm in length and 0.5 mm in diameter).

All of the processes of pellet production follow the standard operating procedure of PT. Widodo Makmur Perkasa.

Nutritional composition of the PCS was 89.24% dry matter (DM), 20.82% crude protein (CP), 8.56% crude fibre (CF), 3.64% ether extracts (EE), 5.58% Ash, and 86% total digestible nutrients (TDN). The nutritional composition of dietary treatments is presented in Table 1.

Regarding the cross over design, there were two periods of experimental phase in which the duration of each phase was 6 weeks. In the first phase, post-weaning calves were assigned to dietary treatment twice a day at 07.00 a.m. and 3.00 p.m. for six weeks then following by two weeks of resting period.

Thereafter, the first and second treatments were exchanged and treated similarly to the first period. Feeding offered for each calf was calculated to meet the requirement of energy and protein.

#### Data collections and sampling

Data were collected for feed intake, feed efficiency, growth, and blood parameters. Feed intake was recorded weekly and determined by calculating the difference between diet supplied and refusal in the feeder trough (by composites). The average daily gain (ADG) was recorded bi-weekly by weighing individual calf (BW gain) and divided by length of the period (42 days; accumulative for one period) then feed efficiency was estimated as the ratio between ADG and DM intake according to Braun et al. (2019). Blood samples were collected in the initial and final period of rearing by sampling 10 mL of blood by jugular venipuncture into heparinized tubes (10 mL, 135 USP U lithium heparin; Venosafe; Terumo Europe NV, Leuven, Belgium), before the morning feeding. The collected samples were immediately transported to the laboratory and analyzed for hematological profile (erythrocyte, leucocyte, lymphocyte, hemoglobin, and hematocrit) by using a hematology analyzer and blood mineral (calcium and phosphorus).

#### **Chemical analysis**

The chemical composition of feed ingredients and experimental diets are presented as % of DM (Table 1). The DM was calculated after oven drying at 55 °C for 72 h and milling through a 1-mm screen (AOAC, 2005; method ID 934.01).

Ash content was determined by combustion at 550 °C for 16 h according to AOAC (2005); (method ID 942.05). Crude protein concentration was calculated by multiplying the N content originated from the Kjeldahl method (ID 988.05; AOAC, 2005) by 6.25. Ether extract content was measured by the method of AOAC (2005).

## Statistical analysis

The data from all observations were included in the statistical analysis. The data were analyzed by two-way ANOVA using the generalized linear model (GLM) procedure within the SAS program (SAS, 2003) according to  $2 \times 2$  factorial design where sex and dietary treatments set as fixed effect and replication (individual animal from all period) as a random effect. Duncan's new multiple range tests were used when P < 0.05 to determine significant differences among the treatments.

# **RESULTS AND DISCUSSION**

#### **Calves performance**

The effect of dietary treatments, sex and their interaction on Intake for dry matter (DM), gross energy (GE), and performance of post-weaning calves is provided in Table (2). No significant effect of diet, sex, and their interaction was observed on DM intake and initial body weight (P>0.05). In all parameters excluding GE intake, no significant difference between male and female treatment was observed. As expected, according to Table (2), PCS supplementation exhibited a positive effect on post-weaning calves' performance (P<0.05). Final body weight, daily gain, and feed efficiency were higher for the group with PCS (P<0.01). These results can be associated with a direct effect from higher energy consumption (GE) in animal fed TMR contained PCS (70.9 $\pm$ 2.94 *vs.* 67.6 $\pm$ 2.71 MJ/d).

## Hematological profile

The hematological profile of post-weaning calves offered different dietary treatment is presented in Table (3). According to the table, there was no significant difference in the value of red blood cells (RBC), white blood cells (WBC), hematocrit, leucocyte, and lymphocyte of calves between PCS and CON group (P>0.05). However, dietary PCS contributed to increasing the value of hemoglobin of calves as observed in the final period (P<0.05). The pattern of red blood cells (RBC) showed that feeding PCS resulted in lower value on the final RBC compared to the initial value. There were no significant differences between the treatment of RBC value (P>0.05). The range of RBC was between 8.53-9.01 10<sup>6</sup>/mm<sup>3</sup> in the initial period and 7.89-8.54 10<sup>6</sup>/mm<sup>3</sup> in the final period, respectively.

#### Plasma mineral concentration

In our study, the range of Ca plasma concentration in all experimental groups collected over in the initial and final period was 10.56-13.38 mg/dL (Table 4). According to NRC (2001), the normal range of serum Ca concentration is above 9.0 mg/dL, indicated that Ca concentration for both groups in the current study was in the normal range.

Table 1 Feed ingredients and nutrient composition of dietary treatments for crossbreed post-weaning calves

T	Dietary	treatments
Ingredients and nutrient composition	Control	Treatment
Feedstuff component	(% of tot	tal amount)
Napier grass	10.0	10.0
Rice straw	4.0	4.0
Concentrate	83.4	63.4
PCS	0.0	20.0
Molasses	2.4	2.4
AGROMIX Booster® <sup>1</sup>	0.2	0.2
Amount	100.0	100.0
Nutrient contents	(In 10	0% DM)
Crude protein (%)	9.17	10.92
Extract ether (%)	2.72	2.83
Crude fiber (%)	16.78	14.04
Ash (%)	11.17	9.90
Nitrogen-free extract (NFE) (%)	60.16	62.31
Total digestible nutrient (TDN) $(\%)^2$	68.51	78.28
Gross energy (MJ/kg DM) <sup>3</sup>	18.31	18.34

<sup>+</sup> AGROMIX *Booster®* contains= Ca: 26.45%; P: 0.62%; K: 0.22%; Mg: 0.26%; Na: 4.70%; S: 681.7 ppm; Fe: 1.44%; Zn: 300.2 ppm; Cu: 229.7 ppm; Se: 0.541 ppm; Co: 7.7 ppm; vitamin A, D, E and K, probiotics (*L. acidophilus, Enterococcus faecium, Saccharomyces cerevisiae*) and blended essential oils mixture from extracted walnuts, olives, soybeans, lavender, eucalyptus, coconut, orange, peppermint and sesame seeds.
 <sup>2</sup> TDN and Gross energy were calculated according to equations from Hartadi *et al.* (1980).

Table 2 Effect of PCS supplementation, sex and their interaction on intake and performance of crossbreed post-weaning calves

Variables	Diet (D) Control Treatme		) Sex (S)			Significance		
variables			ent M F		D S		S $D \times S$	
DM intake (kg/d)								
DM (kg/d)	3.7±0.40	3.9±0.46	3.7±0.52	3.9±0.31	NS	NS	NS	
GE (MJ/d)	$68 \pm 2.7^{b}$	71±2.9ª	67±1.9 <sup>b</sup>	$71\pm2.2^{a}$	*	*	NS	
Performance								
Initial weight (kg)	99±6.3	105±5.9	104±6.1	99±4.3	NS	NS	NS	
Final weight (kg)	169±7.2 <sup>b</sup>	198±10.2 <sup>a</sup>	186±8.4	180±9.2	**	NS	NS	
Daily gain (kg)	70±3.4 <sup>b</sup>	93±4.3ª	81±3.8	80±3.7	**	NS	NS	
ADG (98 d)	$0.7{\pm}0.01^{b}$	0.9±0.01ª	0.8±0.02	$0.8 \pm 0.01$	**	NS	NS	
Feed efficiency	$0.2 \pm 0.01^{b}$	0.3±0.01ª	0.2±0.01	$0.2 \pm 0.01$	**	NS	NS	

DM: dry matter; GE: gross energy; ADG: average daily gain; D: diet; S: sex; M: male; F: female and D × S: interaction effect between diet and sex.

The means within the same row with at least one common letter, do not have significant difference (P>0.05). \*\* (P<0.01) and \* (P<0.05).

NS: non significant.

Tahla 3	Effect of PCS	supplementation,	sev and their	interaction on	hematological	indices of	crossbreed r	ost_weaning	calves
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<b>X7</b> • 11	Diet (D)		Sex	Significance			
Variables	Treatment	Control	Male	Female	D	S	$\mathbf{D} \times \mathbf{S}$
<b>RBC</b> (10 <sup>6</sup> /mm <sup>3</sup> )							
Initial	9.0±1.9	8.5±2.3	8.6±2.4	8.9±1.9	NS	NS	NS
Final	7.9±1.2	8.5±1.3	8.2±1.5	8.2±1.1	NS	NS	NS
WBC (10 <sup>6</sup> /mm <sup>3</sup> )							
Initial	$10{\pm}2.8$	11±4.1	11±3.6	10±3.3	NS	NS	NS
Final	11±2.2	11±2.3	11±2.0	11±2.6	NS	NS	NS
Hematocrit (%)							
Initial	31±6.9	29±7.5	30±7.8	31±6.7	NS	NS	NS
Final	34±10.8	33±3.2	35±10.7	32±3.0	NS	NS	NS
Hemoglobin (g/dL)							
Initial	10.6±2.2	10.1±2.2	10.3±2.1	10.4±2.4	NS	NS	NS
Final	10.6±1.1 <sup>b</sup>	11.3±1.1ª	10.9±1.2	10.9±1.0	*	NS	NS
Lymphocyte							
Initial (10 <sup>3</sup> /µL)	5.2±1.5	5.6±2.5	5.6±1.94	5.2±2.2	NS	NS	NS
Final $(10^3/\mu L)$	6.5±1.8	6.9±2.6	6.9±1.80	6.5±2.3	NS	NS	NS

RBC: red blood cells; WBC: white blood cells; D: diet; S: sex and D × S: interaction effect between diet and sex.

The means within the same row with at least one common letter, do not have significant difference (P>0.05).

\* (P<0.05).

The p content of all experimental groups was ranged between 7.56 and 8.31 mg/dL. The concentrations of Ca and P were not influenced significantly by experimental diet, sex, and their interaction (Table 4; P>0.05). Feeding PCS containing mineral mix supplementation gave a consequent on higher Ca and P concentration in the dietary treatment in comparison to CON. No significant difference in Ca and P contents were observed between dietary treatments (P>0.05).

Dry matter intake (DMI) among experimental treatments was similar. The findings were similar to studies conducted by Moriel et al. (2018) who reported that similar DMI was mainly caused by similar feedstuffs, palatability, breed, and physiological condition. No difference in DMI, final body weight, daily gain, and feed efficiency between male and female treatment indicate that in the growing period, they have a similar pattern on eating behavior and metabolism since the sex hormone is not fully worked in that age. Hizli et al. (2018) also reported that diets containing similar nutrient composition tend to not affected DMI but might influence final BW due to different energy content and additional factors such as additive inclusion. A similar DMI in the current finding was in contrast with Hashemipour et al. (2013) and Froehlich et al. (2017). Probiotic is known to increase palatability because it modifies nutrient compositions during fermentation. However, the effect on palatability is also varied among different conditions and animal species. Avasan et al. (2006) reported no effect of probiotics inclusion on feed intake of Japanese quails.

Bioactive compounds of EO are known to have antimicrobial properties that facilitated greater DMI (Table 2) and may improve nutrients utilization outputted to higher animal productivity. Nevertheless, it should be noted that the effects vary among different EO sources and may give different responses. For instance, cinnamaldehyde and garlic oil supplementation resulted in no benefit in improving the productivity of pre-weaning and post-weaning growth of calves (Moriel *et al.* 2018), steers (Beck *et al.* 2017), and heifers (Chapman *et al.* 2017). Therefore, it can be speculated that the incorporation of AGROMIX Booster® in the PCS group did not affect DMI due to the different composition of probiotics and EOs compared with other studies.

The energy in the diet is the most essential constituent that determines the productivity of ruminants since it plays in a major process of metabolism (Moriel *et al.* 2018). Higher final body weight of calves receiving PCS is greatly correlated with the composition of the PCS which is containing probiotics and essential oils (EOs) mixed. Probiotic is known to alter gut microbiota by balancing the population of beneficial bacteria and help to increase nutrients degradability thus improving feed efficiency and animal performance.

Kelsey and Colpoys (2018) reported that probiotics inclusion significantly resulted in greater ADG, feed efficiency, and final BW of weaned calves. Another evidence was also reported by Karimi-Kivi *et al.* (2015) who testes several commercial probiotics and found an improvement in the growth performance, body characteristics, and hematological parameters.

On the other hand, there are a number of studies showed beneficial effect of EOs supplementation as reported in improving the immune system and animal performance (Jamshidparvar *et al.* 2017; Ornaghi *et al.* 2017; Soltan *et al.* 2018), although the effect is doses and EO-types dependent.

For instance, in another animal study, Ayasan (2013) also found no effect of essential oils from *Yucca schidigera* on hatchability and fertility of Japanese quails egg. It has confirmed that active compounds of EO can be antibiotics alternative because they have antimicrobial properties (Cruz *et al.* 2014). Essential oils addition favorably modifies rumen fermentation by several modes of actions, including by inhibiting methanogens, protozoa, and other undesirable microbes thus produce lower methane emission and higher volatile fatty acids (VFA) as a source of energy (Ornaghi *et al.* 2017).

Decreasing methane production in the rumen is a way to reduce energy losses (Castro-Montoya *et al.* 2015). The further beneficial effect when VFA increases is the enhancement of microbial protein synthesis and improvement of nitrogen use efficiency, leading to higher animal productivity (Soltan *et al.* 2018). Salazar *et al.* (2019) also compared the efficacy of some feed additives and found that EO had a greater effect in improving feed efficiency, health status and animal performance than the control diet. When correlated to the current findings, these mechanisms suggested that probiotics and EOs in the AGROMIX Booster® have a synergistic effect on post-weaning calves.

Increasing the hemoglobin value in the treatment group agreed with Kumar *et al.* (2018) who reported improvement in the hematological profile of crossbred calves fed *Aloe barbadensis* and Czech *et al.* (2018) by adding fodder yeast in the piglet.

The increase in hemoglobin is attributable to antioxidant content and other bioactive compounds of essential oil contained in the PCS. The bioactive component was suggested to have a stimulating effect on hemoglobin synthesis and red blood cell production. Hashemipour *et al.* (2013) explained that the antioxidant activity of herbs and spices often originates from their phenolic compounds inherent in herbal EO. There are some major chemical components in EO originated from thyme, eucalyptus, and celery; thymol, citronellal, and phthalides (Benchaar *et al.* 2007), in which relevant to the EO content of current study.

Variables	Diet (D)		Sex	Significance			
variables	Treatment	Control	Male	Female	D	S	$D \times S$
Ca (mg/dL)							
Initial	12.1±4.11	11.6±2.03	$13.4 \pm 3.62^{a}$	10.3±1.77 <sup>b</sup>	NS	**	NS
Final	10.5±0.90	10.6±0.93	10.5±0.80	10.7±1.01	NS	NS	NS
P (mg/dL)							
Initial	7.9±1.25	7.7±1.46	8.1±1.06	7.6±1.25	NS	NS	NS
Final	8.1±1.08	8.1±1.60	8.3±1.64	7.9±0.97	NS	NS	NS

 Table 4
 Effect of pelleted-concentrate supplement (PCS) supplementation, sex and their interaction on plasma calcium and phosphorus concentration of crossbreed post-weaning calves

D: diet; S: sex and D  $\times$  S: interaction effect between diet and sex.

The means within the same row with at least one common letter, do not have significant difference (P>0.05). \*\* (P<0.01)

NS: non significant.

Yeast probiotics are known as a valuable source of easily digestible micronutrients, including iron and copper, i.e. elements taking part in the synthesis of red blood cells (Czech *et al.* 2016), which are particularly important in young animals (Kumar *et al.* 2012). In regard to the increase in hemoglobin value, it may also be attributed to the fact that fodder yeast, in this study was probiotics, is a valuable source of  $\beta$ -glucans which can stimulate the production of cytokines and intestinal lymphocytes through activation of Peyer's patches, leading to an increase in mucosal immunity (Kogan and Kocher, 2007; Volman *et al.* 2008). This stimulatory effect has also been confirmed in many studies in which the probiotics exhibited a positive effect on immune modulation in various animal species (Czech *et. al.* 2018).

The range of RBC was in the normal range according to the reference (Klem et al. 2010). The normal value of RBC indicated that the blood of the calves had enough carrying ability to oxygen for normal metabolism system and also had no risk of anemia incidence (Brian, 2009). In comparison to other studies, there were contrary results of RBC in the current study. Czech et al. (2018) found a simultaneously changed on RBC, WBC, and hematocrit in the group of animals received supplemented diet containing fodder yeast i.e. Saccharomyces cerevisiae and Yarrowia lipolytica. In regard to other hematological parameters such as RBC, WBC, hematocrit, and lymphocyte, no effect of PCS inclusion was found in this work meaning that probiotics and EOs used in this study had no effect on immune functions. Jamshidparvar et al. (2017) and Karimi-Kivi et al. (2015) reported contrasting results whereas probiotics and EOs from Heracleum persicum Desf showed a positive effect on immune and haemotological profile. This difference response could be associated with different compositions of probiotics and animal species. Probiotics, in the metabolism system, influences lower bile salts to recycle inhibit the activity of hepatic 3-hydroxy-3and methylglutaryl coenzyme A. In addition, active compounds of EOs can stimulate enzyme activity and secretion in the gut system and also humoral and cellular immune system (Jamshidparvar et al. 2017).

Results of the present work indicated that beneficial effects of AGROMIX Booster® are largely influenced by its role in modulating rumen fermentation rather than improving the immune system of the calves.

Calcium (Ca) and phosphorus (P) are two major important minerals that play an important role in many biological processes, primarily in post-weaning calves for body development and active metabolism. The range value of Ca and P in this study was in agreement with the value obtained by Bhatti *et al.* (2012). No difference in Ca and P content between dietary treatments indicated that PCS has limited effect and without incorporation of PCS, the incidence of metabolic diseases correspond with Ca deficiency was scare. It has been also reported that Ca or P supplementation often resulted in similar animal performance when the between the group of treatment has low variation. For instance, Carrasco *et al.* (2012) suggested that post-weaning calves received calcium propionate as additives resulted in equal growth performance.

Regarding the effect on Ca concentration, it is difficult to conclude because of limited references related to the specific requirement of male or female calves. Large variation of data can be one of the associated factors. Since the difference only appeared in the initial period, it can be related to the previous metabolic status of the calves. Farnia *et al.* (2018) suggested when cattle experience a mineral deficiency; they use mineral storage in the body including calcium to be utilized in the metabolism process. A higher concentration of Ca in the male group may be associated with the incidence of Ca deficiency in the initial period when an excessive amount of Ca is utilized and metabolized; the amount in the blood plasma will be higher. This can be seen in the initial BW of male calves that have lower BW compared to female calves.

# CONCLUSION

This study concluded that inclusion of 20% PCS into TMR based diet improved feed utilization efficiency and the calves performance such as higher daily gain and final body weight. The beneficial effect can be associated with the

synergistic effect from probiotics and essential oils contained in the PCS. The inclusion of PCS had no effect on blood composition profile and plasma calcium and phosphorus concentrations. Results of the present work indicated that beneficial effects of AGROMIX Booster® incorporated in the PCS are largely facilitated by its role in modulating rumen fermentation rather than improving the immune system of the calves.

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