Zinc-methionine supplementation prevents the live weight loss in the early lactation of Ongole-Crossbred cows in the reproduction cycle

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Abstract. This research aimed to investigate the reproductive performance of Ongole crossbred (PO) cows offered zinc methionine (ZincMeth) during the late gestation and early lactation. Thirty-two PO pregnant cows, 400 kg live weight (LW) were weighed and measured body condition score (BCS) before the experiment. The experimental design was a 2 x 2 factorial with two nutritional and two ZincMeth treatments, with eight cows in each nutrition x ZincMeth group. All cows were offered elephant grass (EG), 16 cows offered a concentrate diet (CONS) containing 15% crude protein (CP) and 16 cows offered a CONS containing 11% CP at 3% LW on a DM basis, the ratio of EG: CONS was 40:60. Five grams of ZincMeth was added to the CONS prepared for individual cows. The parameters observed were LW gain, BCS, post-partum anestrus period, luteinizing hormone, volatile fatty acid, and blood metabolite concentrations. The results showed that the BCS change was greater in cows fed a high CP diet, they also had high plasma glucose, urea, and total protein concentrations. ZincMeth prevented LW loss in the early lactation and increased plasma urea concentration. Thus, the ZincMeth maintained the LWG during the critical period but did not affect any reproductive parameters.

1. Introduction

The low reproductive performance of cows is usually affected by low nutrient intakes especially in two critical periods, in the late pregnancy and the early lactation. Before calving, cows require more nutrients for maintaining the pregnancy as well as preparing for parturition. Nutrient intake affects the body condition score (BCS) that is thought to be an important factor for the normal cycle of estrus [1]. Besides, cows that do not eat enough nutrients may suffer from negative energy balance in early lactation due to energy expenditure associated with high milk production for the calves. This results in a high lipid mobilization from body fat depots [2]. Therefore, nutrition is an important factor that affects the BCS and reproductive performance of cows.

Protein and energy are two limiting factors that have to be supplied for cows. However, cows also require minerals beside those two fractions. Minerals such as Zinc (Zn) involves in many biochemical reactions in the body of animals [3,4]. There are some roles of Zn in the reproductive performance of cows, El-Nour [5] showed that Zn shortened the post-partum anoestrous interval (PPAI) at around 43 days in comparison with the untreated group (around 54 days) in lactating goats. Supplementation
with Zn also improves the uteri involution after calving and increases the size of follicles [6]. Also, zinc can prevent the damage of the reproduction track caused by mycotoxin in the grain that is usually available in the ruminant feed [7]. Therefore, zinc is critical in the reproductive system of animals.

However, the source of Zn affects the readily available Zn for the body, Mir et al. (2020) reported that organic Zn tends to be more absorbed in comparison with inorganic ones [8]. Similarly, organic Zn showed higher retention and bioavailability both in poultry and ruminants [9]. Whereas the common Zn is supplemented in the diet of animals as inorganic salts that were reported insufficiently absorbed and less retained in the tissues. Thus, organic Zn has a potency for ruminants’ feed. The current study aimed to investigate the reproductive performance of Ongole crossbred cows offered EG grass and the CONS of a diet supplemented with organic zinc.

2. Methods
The following experiment was conducted under the guidelines of the Indonesian Code of Practice for the Care and Use of Animals for Scientific Purposes and was approved by the Indonesian Ministry of Agriculture Animal Ethics Committee (Balitbangtan/Lolitsapi/Rm/10/2018).

The experiment consisted of a 14-day pre-experimental period followed by a 160-day experimental period. The experiment commenced in early April 2018 and was completed in early December 2018. Before the start of the experiment, 36 Ongole crossbred pregnant cows of approximately 400 kg LW were selected from the herd of the population in Indonesian Beef Cattle Research Station (BCRS) at Grati, East Java, Indonesia, and were weighed and measured (LW and BCS) before the experiment was conducted.

| Ingredients (%)             | Concentrate |         |
|-----------------------------|-------------|---------|
|                             | A           | B       |
| Wheat pollard               | 15          | 30      |
| Ground cassava              | 15          | 38      |
| Palm kernel cake            | 12          | 17      |
| Copra meal                  | 15          | 8       |
| DDGS                        | 15          | 2       |
| Commercial CONS             | 26          | 3       |
| Salt                        | 1           | 1       |
| Limestone                   | 1           | 1       |
| Total                       | 100         | 100     |

Chemical composition of feed offered to cows (mean ± SEM)

|                          |              |              |
|--------------------------|--------------|--------------|
| Dry matter (%)           | 92.9±0.09    | 92.5±0.04    |
| Crude protein (% DM)     | 15.0±0.00    | 11.3±0.28    |
| NDF (% DM)               | 35.1±0.33    | 37.0±1.99    |
| ADF (% DM)               | 28.1±0.03    | 20.1±0.89    |
| TDN (% DM)               | 79.0±3.96    | 71.8±4.98    |

DDGS: dried distillers’ grains with solubles, NDF: neutral detergent fiber, ADF: acid detergent fiber, TDN: total digestible nutrient.

A 2-week pre-experimental period consisted of two-week feeding in individual pens, with 1 cow/pen. Cows were fed fresh elephant grass (*Pennisetum purpureum*; 893 g OM, 70 g CP, 712 g NDF /kg DM) with unrestricted access to fresh drinking water throughout the pre-experimental period. After the pre-experimental period, 32 cows were selected for inclusion in the experiment based on temperament, eating behaviour, LW, and BCS. The experiment began 60 days before the expected date of calving and continued during 100 days of lactation, therefore, the measurement period was 35 weeks in total.
The experimental design was a 2 x 2 factorial with two nutritional treatments (A and B) and two organic zinc treatments (+Zn and –Zn), with eight (8) cows in each nutrition x organic zinc treatment group. The two nutritional treatments were shown in Table 1.

All cows were offered EG and CONS diet at 3% LW on a DM basis, with the ratio of EG: CONS diet was 40:60. Before the commencement of the experiment, the 32 cows (390±30 kg LW, mean ±SD) were ranked and blocked on LW. Cows were allocated to individual pens with one cow in each pen. Within each block, two cows were randomly allocated to each of the dietary treatments and then one cow from each dietary treatment was randomly allocated to either + organic zinc or -organic zinc treatments.

The organic zinc treatment used in this study was zinc-methionine produced by the Indonesian Research Institute for Animal Production (IRIAP)’s laboratory. The dose of ZincMeth was 5 g/head/day. ZincMeth was supplied in the feed trough for every cow treated with the supplement at the same time as feeding the CONS diet so that the cows licked.

2.1. Measurements

2.1.1. Feeding and feed intake. Cows were fed at 07.00 AM each morning after refusals from the previous day were collected and weighed. The cows were offered 10% above the average intake of the previous week on an as-fed basis. Feed intake was measured daily throughout the experimental period by subtracting the amount of feed refused from the weight of feed offered. Sub-samples of feed offered were collected each day, bulked for a week mixed thoroughly, and duplicate sub-samples were collected for proximate analysis (DM, CP, organic matter (OM), crude fiber (CF), and total digestible nutrient (TDN) [10].

2.1.2. Liveweight gain, body condition score, and blood collection. Liveweight was measured prior to feeding once per week. Body condition score (BCS) was assessed by the same person at the start and at the end of the experimental period using a 1 to 5 score system (score 1 was emaciated and score 5 was obese) [11,12] which is based on visual assessments of body condition at specific points in the body of the cow, ranging through a scale of 1 to 5, with subunits of 0.25 points, regardless of the cow weight or size (i.e., height, thoracic girth, length). Blood samples were collected before feeding before the experimental period, at day 21, 40, 60, and 90 after calving for luteinizing hormone (LH) and blood metabolite analyses.

2.1.3. Rumen characteristics. The concentration of NH3, acetic acid, propionic acid, butyric acid and the pH from the rumen liquor were measured on week 20 by collecting rumen fluid at 4 hours after eating.

2.1.4. Statistical analysis. The SPSS software program (SPSS Statistic, IBM, New York) version 23 with the General Linear Model was used to analyse animal measurement data. The SPSS Mixed Model procedure was used to analyse luteinising hormone data that were measured repeatedly. Nutritional, time of blood collections, and all of the interactions were included in the model as fixed variables, and cows within a treatment were included as random factors. The data presented in the bar chart were least-square means. The 5% level significance was used to consider the difference between means.

3. Results and discussion

3.1. Feed intake, live weight gain, and body condition score

The treatments were not significantly different neither on DM intake nor LWG in the late pregnancy as well as in the early lactation (table 4). Although the A group had a slightly higher DM intake, it was not different between the treatment, similarly, the increased of LW throughout the experiment was
also not different between dietary treatment. Our results demonstrated that although a low protein diet was fed to the cows during the late pregnancy period, it did not decrease the LWG as far as the DM intake was enough. Dry matter intake is more likely affected by the NDF content of the diet [13], our results indicated that the NDF content between the two different CONS diets was similar (table 1). Feeding EG also meet enough energy for the productive performance of Ongole cows, these results were in line with Antari et al (2014) who also found that Ongole crossbred cows fed EG had BCS greater than 3 and EG provided enough energy for cows to maintain sufficient BCS especially for successful reproduction [14]. Early lactation became the critical time when cows would enter the next oestrus cycle. Therefore, maintaining the LW and BCS is very important during those critical periods.

Cows in our experiment had an average starting weight of 392 kg and were in BCS 2.8 on a 1–5 scale. To increase the reproductive performance of Ongole cows would need to have a BCS of 3 or higher [15]. The current results suggested that using the diet in the current experiment increased the BCS quickly. The implication of the increasing BCS might reduce the postpartum anoestrus intervals and hence possibly reduce calving intervals is possible.

Feeding ZincMeth prevented cows from losing the LWG during early lactation. However, the supplement did not affect DM intake, LWG in late pregnancy, nor change in BCS. Although, the previous experiment did not result in a significant difference in BCS or BCS change of dairy cows fed organic minerals both in pre- and post-partum [16]. The difference probably due to the experience of the operators in judging the BCS. Although, we already minimised the limitations by using three people doing BCS judgment. No diets x zinc organic effects were detected (P>0.05) on the parameters observed.

### 3.2. Rumen characteristics

No concentrate diet and ZincMeth effects were detected (P>0.05) on mean rumen NH3, acetic acid, propionic acid, butyric acid, and the pH. Butyric acid only approached significant (P=0.06) on the concentrate diet effect while the pH approached significant on both CONS diet and zinc-methionine supplement (table 3). Although, the concentration of acetic, propionic, and butyric acids in A concentrate diet and ZinMeth supplemented cows were slightly higher than B cons diet and non-ZinMeth supplemented cohorts. No concentrate diet and ZincMeth supplement interactions were detected in all rumen parameters observed (P>0.05).

### Table 2. Dry matter intake (DMI), live weight gain (LWG), and the final body condition score (BCS) of Ongole crossbred cows fed different nutritional treatments and either offered organic zinc or not over 32 weeks.

|                | Concentrate Diet (D) | Zinc Methionine (ZM) | DxZM |
|----------------|----------------------|----------------------|------|
|                | A                    | B                    | SEM  | P     | (+) Zinc Meth | (-) Zinc Meth | SEM  | P     | P     |
| DM intake (% W/d) | 2.7                  | 2.6                  | 0.08 | 0.38  | 2.7           | 2.6           | 0.08  | 0.59  | 0.21  |
| LWG (kg/d)      | 0.8                  | 0.6                  | 0.07 | 0.11  | 0.8           | 0.8           | 0.08  | 0.30  | 0.85  |
| - late pregnancy | 0.1                  | 0.03                 | 0.06 | 0.38  | 0.19<sup>a</sup> | -0.06<sup>b</sup> | 0.06  | 0.004 | 0.37  |
| Change in BCS   | 0.6                  | 0.3                  | 0.04 | >0.001 | 0.47          | 0.49          | 0.05  | 0.78  | 0.75  |
| (1-5 scale)     |                      |                      |      |        |               |               |       |       |       |

<sup>1</sup>See materials and methods for the description of nutritional treatments: A= concentrate diet containing 15% crude protein, B= concentrate diet containing 11% crude protein. <sup>2</sup> were offered at the same time as feeding concentrate diet every day throughout the experiment. <sup>3</sup>Values are least-square means with a standard error of the mean (SEM); different superscripts across a row within the diet and Zinc-methionine main effects indicate significantly different treatment means (P≤0.05).
Our results of acetic acid concentration were lower than those observed by Spears [17] but comparable to Wang et al. [18], they found that the acetic acid concentration of steers supplemented with organic zinc was 64 mol/100 mol. They also said that the acetic acid concentration in steers offered ZincMeth was lower than inorganic zinc sources, this was probably the cause of low acetic acid concentration in our cows. Similarly, the propionic and butyric acids were also low. The low concentration of VFA probably related to a slower rate of feed consumption or a reduced rate of ruminal digestion spears 2004. A possible limitation of our study was no bioavailability data was measured, Spears [19] showed that usually organic minerals are more bioavailable and readily absorbed than inorganic sources. Interestingly, the ratio of forage to concentrate diet more likely affected the concentration of VFA in rumen liquor, while in the current experiment the ratio of forage to CONS diet was all the same.

Table 3. The concentration of NH$_3$ (mg/100 mL), acetic acid (mmol), propionic acid (mmol), and the pH in the rumen liquor of Ongole cross breed cows fed different nutritional treatments$^1$ and added organic zinc or not over 32 weeks$^2$.

| Rumen Parameters | Concentrate Diet (D) | Zinc Methionine (ZM) | DxZM |
|------------------|----------------------|----------------------|------|
|                  | A  | B     | SEM | P  | (+) | Zinc Meth | SEM | P  | (+) | Zinc Meth | SEM | P  |
| NH$_3$           | 8.5 | 8.9  | 0.29 | 0.84 | 9.1 | 8.5  | 0.29 | 0.89 | 0.22 |
| Acetate          | 32.7 | 25.4 | 2.2  | 0.08 | 29.3 | 25.1 | 2.34 | 0.58 | 0.64 |
| Propionate       | 20.2 | 15.4 | 1.45 | 0.16 | 15.9 | 14.7 | 1.47 | 0.15 | 0.40 |
| Butyrate         | 13.6 | 9.7  | 1.04 | 0.06 | 10.9 | 9.0  | 1.10 | 0.27 | 0.52 |
| pH$^3$           | 6.6 | 6.5  | 0.03 | 0.06 | 6.7  | 6.5  | 0.56 | 0.06 | 0.23 |

$^1$See materials and methods for the description of nutritional treatments: A= concentrate diet containing 15% crude protein, B= concentrate diet containing 11% crude protein. $^2$ were offered at the same time as feeding concentrate diet every day throughout the experiment. $^3$Values are least-square means with a standard error of the mean (SEM); different superscripts across a row within the diet and additional organic zinc main effects indicate significantly different means ($P \leq 0.05$). $^3$pH measurement was taken 4 hours after eating.

3.3. Luteinising hormone profile, estrus detection, and post-partum anoestrous interval (PPAI)
The luteinising hormone was measured on days -21, -40, -60 and -90 after calving. On days -21 and -60, the concentrations of LH were below 20 ng/mL that was not different between treatments. Timepoint affected the concentration of LH, figure 1 showed the concentration of LH was significantly higher on day -40 ($P<0.001$), but did not differ between treatment, while on day -21, -60 and -90, the concentration of LH did not show a significant difference ($P>0.05$). The test of within-subjects effects on time x treatment was not different ($P>0.05$).

Our results on LH concentration were comparable to the previous studies [20-22] ranging from 7 to 100 ng/mL during oestrus, the variation probably because of breed differences, nutritional treatments, or oestrus peak time. Our results may have limitations because the pulsatile release of LH is regulated by gonadotropin-releasing hormone (GnRH) that varied hourly during the peak of the oestrus cycle, although we minimised the variation by taking the blood samples at the same time (at 08.00 am). Therefore, we could not detect whether at 08.00 AM sample collections were at pre-oestrus, oestrus or met-oestrus.

During the experimental period, cows were not mated. Because the cows were placed in the individual pens, therefore visual detection was the only way of oestrus detection. Oestrus detection was observed by simply looking for the changes in animal behaviour and the changes in vulva appearance (secreting mucous, the heat rise, and red and swelling vulva). Usually, oestrus can be easily detected with a cow standing to be mounted by a bull or another female. After calving, the normal physiological process is ovarian inactivity that is characterised by a slow follicular growth rate, no oestrus that could be detected, and no response to GnRH called post-partum anoestrous. Studies
reported that *Bos indicus* cows more often show long post-partum anoestrus due to low BCS at calving, very good mothering ability, and nutritional deficiency in early lactation [23,24].

The results showed that no CONS diet and ZincMeth effects on oestrus, the length of anoestrus post-partum in cows fed A CONS diet was (73.2 days) and B counterparts were 91.9 days but did not show a significant difference (P=0.16). While the oestrus length in cows supplemented with ZincMeth was 69.9 days and cows that were not offered ZincMeth was 76.5 days. Fifty percent of cows fed a high protein diet underwent oestrus <60 days. However, ZincMeth supplementation only contributed to 37% of cows that underwent oestrus <60 days. Although, all cows in the current experiment experienced estrus <90 days post-partum, which were shorter than those in the villages in East Java, about >150 days [25].

### 3.4. Blood metabolite characteristics

The concentrations of blood metabolites were measured because it showed the biochemical component that played a significant role in the maintenance of normal physiology. The optimum concentration of blood metabolites is essential for normal body function including the reproduction system. Any changes in the blood metabolites can cause reproductive disorders.

There were CONS of diet effects on mean glucose, urea, and total protein concentration (table 6). Interestingly, no CONS diet effects were detected during the lactation period for urea and total protein (P>0.05). Cows fed a higher protein diet tended to have higher concentrations of glucose, urea, and total protein. The results of the current study were remarkable as it was known that the CONS of diet (A) fed to the cows could increase blood glucose that probably through a process of conversion in the liver from propionic acid. Similarly, studies also found that low protein and or energy diet restriction resulted in low blood glucose concentration [26,27]. Blood glucose is more likely linked between nutritional status and reproductive function at the hypothalamus in beef cattle [28], cows that experienced a negative energy balance showed a reduction in reproductive performance [29]. Therefore, cows fed a low protein diet may accumulate less N per day than is required during the
critical period for fetal development. This suggested that protein must be partitioned from maternal body tissues that affected the change in BCS (table 2).

A lower glucose concentration (P≤0.01) in cows fed the B CONS diet did not show a negative energy balance. Cows experienced a negative energy balance had a low glucose level <30 ng/dL [29]. A low concentration of glucose in the bloodstream may depress the release of GnRH and consequently reducing the frequency of LG pulses to support follicular growth [30]. Therefore, the concentrations of LH in the current experiment were not different between treatments at every time point of sample collection (figure 1).

Table 3. Glucose (mg/dL), urea (mg/dL), and total protein (TP) (g/dL) concentration during pregnancy and lactation in the plasma of Ongole cross breed cows fed different nutritional treatments1 and added organic zinc or not over 32 weeks2.

| Blood metabolites | Diet (D) | Zinc Methionine (ZM) | DxZM | SEM | P (+) | SEM | P (-) | SEM | P |
|-------------------|---------|---------------------|------|-----|-------|-----|-------|-----|----|
| Glucose           |         |                     |      |     |       |     |       |     |    |
| - pregnant        | 64.3a   | 60.0b               | 1.66 | 0.01| 62.5  | 61.0| 1.33  | 0.69| 0.04|
| - lactation       | 56.0a   | 52.0b               | 1.11 | <0.01| 55.0  | 53.0| 0.89  | 0.11| 0.04|
| Urea              |         |                     |      |     |       |     |       |     |    |
| - pregnant        | 18.9a   | 17.2b               | 0.52 | 0.01| 19.2a | 16.9b| 0.43  | <0.01| 0.06|
| - lactation       | 18.5    | 18.8                | 0.27 | 0.34| 19.1a | 18.2b| 0.19  | <0.05| 0.18|
| Total protein     |         |                     |      |     |       |     |       |     |    |
| - pregnant        | 8.0a    | 7.7b                | 0.08 | <0.01| 7.9   | 7.8 | 0.08  | 0.26 | <0.01|
| - lactation       | 7.8     | 7.7                 | 0.04 | 0.24| 7.8   | 7.7 | 0.05  | 0.82 | <0.01|

1See materials and methods for the description of nutritional treatments: A= concentrate diet containing 15% crude protein, B= concentrate diet containing 11% crude protein. 2were offered at the same time as feeding concentrate diet every day throughout the experiment. 2Values are least-square means with a standard error of the mean (SEM); different superscripts across a row within the diet and additional organic zinc main effects indicate significantly different means (P≤0.05).

The differences between cows fed A and B CONS diets in urea and total protein plasma concentration were probably caused by the different levels of protein content in the diet (15 vs 11% CP). However, ZincMeth effects were only detected in blood urea nitrogen both in pregnant (P<0.01) and lactation (P<0.05) periods. Cows supplemented with ZincMeth had a higher urea concentration. Our findings were in contrast to Kinal et al (2007) and Cortinhas et al (2012) who found no supplement effects detected in blood urea concentration [16,31]. There were interactions between diet x ZincMeth supplement in glucose and total protein but was not detected in blood urea nitrogen.

4. Conclusion
The current results found that:
1. The reproductive performance of Ongole crossbred cows offered elephant grass and concentrate containing a high protein diet showed oestrus <60 days (50% of the cows) while organic zinc only contributed to 37% of cows experienced oestrus <60 days.
2. Zinc-methionine supplementation prevented cows from losing LWG during early lactation which was a critical point for cows to entered the next cycle successfully.
3. It is recommended that supplementing cows with Zinc-methionine at 5 g/d could improve the reproductive cycle indirectly by preventing cows undergo live weight loss.

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