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ABSTRACT

The current study aimed to investigate the effect of formic acid, *S. cerevisiae* or the combination of both on the growth performance and serum indices of the Indonesian indigenous crossbred chicken (IICC). Two hundred of the IICC were allotted to four groups including CONT (basal diet exempted from additive), FORMIC (basal diet administrated with 0.2% formic acid), SACCH (basal diet with 0.3% *S. cerevisiae*) and FORSAC (basal diet with the blends of 0.2% formic acid and 0.3% *S. cerevisiae*). Body weight, feed consumption and feed conversion ratio (FCR) were recorded weekly. At week 8, the chicks were blood sampled and slaughtered. Our results showed that feeding formic acid, *S. cerevisiae* or the combination of both resulted in increased ($P < 0.05$) live body weight, accumulative feed intake and income over feed cost of the IICC. The dietary treatments also decreased ($P < 0.05$) proventriculus relative weight of the IICC. The blends of formic acid and *S. cerevisiae* increased ($P < 0.05$) concentration of serum uric acid and decreased ($P < 0.05$) proportion of wings of the IICC. In conclusion, dietary administration with the blends of formic acid and *S. cerevisiae* improved growth performance and feed efficiency of the IICC.

1. Introduction

The Indonesian indigenous crossbred chicken (IICC) has recently attracted more interest from the consumers. As the cross breeds from the Indonesian indigenous roosters and modern female chickens (Isa Brown commercial layer), the IICC possess an outward appearance and characteristics of meat similar to that of native chickens (Pramono, 2006). Through intensive rearing system, the IICC may reach marketing weights (850–900 g) at 8–10 weeks, much earlier than that of indigenous chickens (500 g at the similar age) (Ma'rifah et al., 2013; Sugiharto et al., 2018). To optimize the growth and health performance, antibiotic growth promoters (AGP) has commonly been administrated in the diets of IICC. However, due to the consumer health concern (the risk of resistant antibiotics in humans), the use of AGP in the chicken diets have eventually been prohibited in Indonesia since January 1, 2018. Study shows that in general the withdrawal of AGP from diets adversely affected the productivity and increased the mortality rate in poultry production (Sugiharto, 2016). For this reason, alternative substitutes for AGP are essential for the sustainability of the chicken farming particularly for the IICC.

Organic acids are one of the AGP alternatives, which have widely been used to keep health status and maximize the growth rate of chickens (Sugiharto, 2016). Currently, there are various types of organic acids available in the market, one of them being formic acid. With high acid levels, formic acid can be used as an antibacterial agent to prevent chickens from pathogenic bacteria (Pathak et al., 2017). Formic acid has also been documented able to improve digestibility and productivities of chickens (Hernández et al., 2006; Pathak et al., 2017). Besides organic acids, yeast-based probiotics such as *Saccharomyces cerevisiae* have also been used by farmers to help protecting the chickens from infections. *S. cerevisiae* is reported to maintain health and improve chicken production performance (Sugiharto, 2016). A recent study has also demonstrated the efficacy of prebiotics containing *S. cerevisiae* in broiler chickens growing (Toader et al., 2018). Such type of probiotic is also very easy to obtain as it is widely used to process (ferment) the foods.

Aside from the numerous benefits of formic acid and *S. cerevisiae*, the efficacy of these additives in replacing the role of AGP in chickens remains unconclusive (Sugiharto, 2016). To maximize its benefits on poultry, organic acids have frequently been combined with other

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bioactive compounds. Recent study by Rodjan et al. (2018) documented that the combination of organic acids and probiotics resulted in better villi height and crypt depth of duodenum and decreased *Escherichia coli* and increased the ratio of *Lactobacillus* spp. to *E. coli* in the intestine of broilers. Conversely, Agboola et al. (2015) did not find any synergistic effect of organic acids and probiotics mixture on the production parameters of broilers when compared with the individual treatment with organic acid or probiotic. The absent effect of organic acids and probiotics blend on growth performance was also seen in cockerels (Fatufe and Matanmi, 2011). Several factors may account for the lack of synergistic action between organic acids and probiotics in the latter studies, one of which is the nature of organic acids and probiotics used (*e.g.*, the low acid tolerance of probiotics to extreme low pH values of organic acids). Likewise, the response to the mix of organic acids and probiotics may vary among the species or breeds of poultry. In the present study, formic acid was combined with *S. cerevisiae* which has been shown to have high acid tolerance to formic acid (Guo et al., 2018). Indeed, the dietary treatment of the blends of organic acids and probiotics has never been implemented in the IICC. The objective of the current work was therefore to evaluate the effect of formic acid, *S. cerevisiae* or their combination on the growth performance and serum indices of the IICC.

2. Materials and methods

2.1. In vivo experiment

The experimental procedures were run with comply to the standard rearing and handling of animals declared in law of the Republic of Indonesia number 18, 2009, regarding animal husbandry, health and welfare. A number of 200 IICC (body weight of 37.8 ± 0.29 g) were employed in the current study. The IICC were individually weighed and immediately distributed to one of four experimental groups, each consisting of 50 birds (five replicates with 10 IICC in each). The treatment groups included CONT (IICC fed basal diet without additive), FORMIC (IICC fed basal diet supplemented with 0.2% formic acid, Baymix Latibon Plus ME, Dr. Eckel GmbH, Niederzissen, Germany), SACCH (IICC fed basal diet supplemented with 0.3% *S. cerevisiae*, Mauripan®, PT. Jaya Fermex, Jakarta, Indonesia) and FORSAC (IICC fed basal diet supplemented with the blends of 0.2% formic acid and 0.3% *S. cerevisiae*). Formic acid, probiotic *S. cerevisiae* or the blends of both were added "on top" (at the end of the mixing process) to the basal diets. The basal diets (in mash form) were formulated as starter and finisher diets (Table 1). The formulated diets were exempted from antibiotics, coccidiostat, anti-fungal agents and enzymes. The feeds and water were provided *ad libitum* to chicks throughout the study period.

The IICC were vaccinated with commercial Newcastle disease vaccine (NDV) through eye drop at day 4 and drinking water at week 4. Weekly, the body weight, feed intake and feed conversion ratio (FCR) were determined. At the end of trial (week 8), two chicks per replicate (10 chicks per treatment group) were blood sampled through their wing veins. The blood was collected in tubes containing no anticoagulant and let to clot at room temperature for 2 h. The serum yielded was subsequently frozen until biochemical and antioxidant analyses. Following blood collection, one chick from each replicate (5 chicks per group; the same chicks as blood sampled) was slaughtered (neck-cut), de-feathered and eviscerated. Shortly, the internal organs were taken and weighed (after being emptied). Also, the commercial proportions of the IICC were determined.

2.2. Laboratory analysis

The concentration of total triglyceride in serum was determined based on the enzymatic colorimetric test employing glycerol-3-phosphate oxidase. The enzymatic colorimetric procedure using cholesterol oxidase/p-aminophenazone was adopted to measure the levels of total cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) in the serum of IICC. The serum concentration of total protein was measured by photometric procedure on the basis on the biuret method. The serum level of albumin was assigned by photometric test adopting bromocresol green. The data on serum globulin were acquired from the total protein minus albumin concentration in serum. The enzymatic color test was used to determine the concentration of uric acid in the serum. The biochemical tests were carried out with kits from DiaSys Diagnostic Systems GmbH, Holzheim, Germany. The concentrations of malondialdehyde (MDA) and serum superoxide dismutase (SOD) were determined spectrophotometrically using kits (Sigma-Aldrich, St. Louis, USA). The antibody titer against NDV was measured based on the hemagglutination inhibition (HI) test, and the titers were expressed as geometric mean titers ($\log_2$).

Table 1
Ingredients and chemical compositions of basal diets provided to the Indonesian indigenous crossbred chicken.

Items	Starter (1 to 4 weeks of age)	Finisher (5 to 8 weeks of age)
Ingredients (%):		
Yellow corn	54.8	58.5
Soybean meal	35.7	32.8
Meat bone meal	4.75	2.00
Soybean oil	1.50	3.50
DL-methionine	0.30	0.30
L-lysine	0.20	0.20
Limestone	0.50	0.50
Dicalcium phosphate	1.50	1.50
Premix (mineral-vitamin mix)[a]	0.50	0.50
Salt	0.25	0.25
Calculated compositions (%, unless otherwise noted):		
Metabolisable energy[b] (kcal/kg)	2900	3080
Crude protein	22.0	20.0
Crude fiber	5.50	5.50
Ca	1.00	1.00
P (available)	0.60	0.60
Lysine	1.20	1.20
Methionine	0.60	0.60

[1] Premix (mineral vitamin mix) contained (per kg of diet) of vitamin A 7750 IU, vitamin D3 1550 IU, vitamin E 1.88 mg, vitamin B1 1.25 mg, vitamin B2 3.13 mg, vitamin B6 1.88 mg, vitamin B12 0.01 mg, vitamin C 25 mg, folic acid 1.50 mg, Ca-D-pantothenate 7.5 mg, niacin 1.88 mg, biotin 0.13 mg, BHT 25 mg, Co 0.20 mg, Cu 4.35 mg, Fe 54 mg, I 0.45 mg, Mn 130 mg, Zn 86.5 mg, Se 0.25 mg, L-lysine 80 mg, Choline chloride 500 mg, DL-methionine 900 mg, $CaCO_3$ 641.5 mg, dicalcium phosphate 1500 mg.

[b] Metabolizable energy was calculated on the basis of formula (Bolton, 1967) as follow: 40.81 {0.87 [crude protein + 2.25 crude fat + nitrogen-free extract] + 2.5}.

2.3. Statistical analysis

The study was set up according to a completely randomized design. The data collected from the current study were treated based on analysis of variance (Steel and Torrie, 1997). When the notable differences ($P < 0.05$) were found among the treatment groups, *post hoc* analysis was further conducted using Duncan's multiple-range test.

3. Results

3.1. Performance and internal organs of the IICC

Data on the performances of the IICC are presented in Table 2. At both time of measurements (week-4 and 8), the BW and accumulative FI were higher ($P < 0.05$) in FORMIC, SACCH and FORSAC when compared with that in CONT, whereas FCR was lower ($P < 0.05$) in FORSAC than in other dietary groups. Feed cost per gain was lower

Table 2
Performances of the Indonesian indigenous crossbred chickens.

Items	Experimental groups				SE	*P* value
	CONT	FORMIC	SACCH	FORSAC		
Week 4						
BW, g	236[c]	257[b]	281[a]	281[a]	4.54	< 0.01
Accumulative FI, g	407[c]	436[b]	466[a]	436[b]	4.65	< 0.01
FCR	2.05[a]	2.00[a]	1.92[ab]	1.79[b]	0.05	0.01
Week 8						
BW, g	609[d]	750[c]	830[b]	885[a]	9.33	< 0.01
Accumulative FI, g	1445[c]	1810[b]	1905[a]	1889[a]	16.9	< 0.01
FCR	2.53[a]	2.54[a]	2.41[a]	2.23[b]	0.05	< 0.01
Feed cost per gain, IDR	19,753[a]	19,840[a]	18,773[a]	17,399[b]	352	< 0.01
IOFC, IDR	7608[d]	9134[c]	10,868[b]	12,703[a]	351	< 0.01

CONT: IICC fed basal diet without additive, FORMIC: IICC fed basal diet supplemented with 0.2% formic acid, SACCH: IICC fed basal diet supplemented with 0.3% *S. cerevisiae*, FORSAC: IICC fed basal diet supplemented with the blends of 0.2% formic acid and 0.3% *S. cerevisiae*, SE: standard error, BW: body weight, FI: feed intake, FCR: feed conversion ratio, IDR: Indonesian Rupiah (the national currency for the Republic of Indonesia), IOFC: income over feed cost.
[a,b,c,d]Values in the same row with different letters represent significant differences ($P < 0.05$).

($P < 0.05$) in FORSAC as compared to other dietary treatment groups. The income over feed cost (IOFC) was higher ($P < 0.05$) in the treated than in CONT birds.

Data on the internal organs relative weight of the IICC are listed in Table 3. The relative weight of proventriculus was lower ($P < 0.05$) in the treated than in CONT birds. The relative weight of other internal organs of chicks did not differ ($P > 0.05$) among the groups.

3.2. Serum SOD, MDA, antibody titer against NDV and biochemical parameters of the IICC

In general, there was no difference ($P > 0.05$) in terms of serum concentrations of SOD, MDA and antibody titer against NDV across the IICC in the present study (Table 4). With regard to serum biochemistry, uric acid level in serum was higher ($P < 0.05$) in FORSAC than in CONT and SACCH, but was not different ($P > 0.05$) from that in FORMIC chicks (Table 5). The other serum biochemical variables such as cholesterol profile (total triglyceride, total cholesterol, LDL and HDL), total protein, albumin, globulin, A/G ratio did not significantly differ among the IICC.

Table 3
Internal organs relative weight of the Indonesian indigenous crossbred chickens.

Items (% live BW)	Experimental groups				SE	*P* value
	CONT	FORMIC	SACCH	FORSAC		
Heart	0.48	0.44	0.43	0.46	0.04	0.70
Liver	2.59	2.04	2.32	2.15	0.15	0.08
Proventriculus	0.73[a]	0.58[b]	0.51[b]	0.57[b]	0.41	< 0.01
Gizzard	3.78	3.14	3.30	3.00	0.27	0.23
Spleen	0.26	0.29	0.20	0.25	0.06	0.78
Thymus	0.26	0.22	0.20	0.26	0.05	0.81
Bursa of Fabricius	0.13	0.08	0.08	0.06	0.03	0.32
Duodenum	0.76	0.59	0.61	0.55	0.05	0.08
Jejunum	1.50	1.29	1.27	1.33	0.17	0.76
Ileum	0.85	0.80	0.78	0.84	0.10	0.95
Pancreas	0.33	0.23	0.25	0.27	0.04	0.33

CONT: IICC fed basal diet without additive, FORMIC: IICC fed basal diet supplemented with 0.2% formic acid, SACCH: IICC fed basal diet supplemented with 0.3% *S. cerevisiae*, FORSAC: IICC fed basal diet supplemented with the blends of 0.2% formic acid and 0.3% *S. cerevisiae*, BW: body weight, SE: standard error.
[a,b]Values in the same row with different letters represent significant differences ($P < 0.05$).

Table 4
Serum superoxide dismutase, malondialdehyde and antibody titer against NDV of the Indonesian indigenous crossbred chickens.

Items	Experimental groups				SE	*P* value
	CONT	FORMIC	SACCH	FORSAC		
SOD (U/mL)	15.2	15.4	14.0	14.9	0.55	0.29
MDA (nmol/mL)	7.88	9.48	8.89	8.54	0.86	0.62
Antibody titer against NDV (log_2 GMT)	0.20	0.40	0.60	0.60	0.30	0.45

CONT: IICC fed basal diet without additive, FORMIC: IICC fed basal diet supplemented with 0.2% formic acid, SACCH: IICC fed basal diet supplemented with 0.3% *S. cerevisiae*, FORSAC: IICC fed basal diet supplemented with the blends of 0.2% formic acid and 0.3% *S. cerevisiae*, SE: standard error, SOD: serum superoxide dismutase, MDA: malondialdehyde, NDV: Newcastle disease virus, GMT: geometric mean titers.

Table 5
Serum biochemical parameters of the Indonesian indigenous crossbred chickens.

Items	Experimental groups				SE	*P* value
	CONT	FORMIC	SACCH	FORSAC		
Total triglyceride (g/dL)	51.5	53.7	43.4	48.4	5.54	0.58
Total cholesterol (g/dL)	131	139	113	132	9.66	0.28
LDL (g/dL)	22.3	34.1	16.9	31.3	6.79	0.21
HDL (g/dL)	101	109	83.8	94.7	6.60	0.07
Total protein (g/dL)	4.32	4.70	4.58	4.74	0.15	0.22
Albumin (g/dL)	1.51	1.56	1.49	1.59	0.05	0.39
Globulin (g/dL)	2.81	3.14	3.10	3.15	0.12	0.18
A/G ratio	0.54	0.50	0.49	0.51	0.02	0.19
Uric acid (g/dL)	5.75[b]	6.76[ab]	5.89[b]	8.08[a]	0.58	0.03

CONT: IICC fed basal diet without additive, FORMIC: IICC fed basal diet supplemented with 0.2% formic acid, SACCH: IICC fed basal diet supplemented with 0.3% *S. cerevisiae*, FORSAC: IICC fed basal diet supplemented with the blends of 0.2% formic acid and 0.3% *S. cerevisiae*, SE: standard error, LDL: low-density lipoprotein, HDL: high-density lipoprotein, A/G ratio: albumin to globulin ratio.
[a,b]Values in the same row with different letters represent significant differences ($P < 0.05$).

Table 6
Carcass traits of the Indonesian indigenous crossbred chickens.

Items	Experimental groups				SE	*P* value
	CONT	FORMIC	SACCH	FORSAC		
	(% live BW)					
Eviscerated carcass	59.4	54.9	54.6	59.4	4.56	0.79
	(% eviscerated carcass)					
Breast	22.3	20.7	19.9	21.2	1.35	0.65
Thigh	16.2	16.8	16.0	16.5	0.37	0.42
Drumstick	16.7	17.3	16.7	17.7	0.48	0.36
Wings	16.1[a]	17.0[a]	16.5[a]	14.4[b]	0.48	0.01
Back	28.8	28.2	30.9	30.1	1.78	0.68

CONT: IICC fed basal diet without additive, FORMIC: IICC fed basal diet supplemented with 0.2% formic acid, SACCH: IICC fed basal diet supplemented with 0.3% *S. cerevisiae*, FORSAC: IICC fed basal diet supplemented with the blends of 0.2% formic acid and 0.3% *S. cerevisiae*, SE: standard error, BW: body weight.
[a,b]Values in the same row with different letters represent significant differences ($P < 0.05$).

3.3. Carcass traits of the IICC

Compared to other treatment groups, the IICC in FORSAC group had lower ($P < 0.05$) proportion of wings (Table 4). The proportions of eviscerated carcass and other commercial cuts (breast, thigh, drumstick and back) of the IICC were not different ($P > 0.05$) among the dietary treatment groups.

4. Discussion

The retraction of antibiotics from diets has widely been attributed to the retarded growth rate and the compromised health status of poultry (Sugiharto, 2016). Data in this study clearly showed that the application of formic acid, *S. cerevisiae* or the combination of both resulted in increased live body weight, accumulative feed intake and income over feed cost of the IICC. In agreement with our findings, Hernández et al. (2006) and Pathak et al. (2017) previously documented the benefits of formic acid in improving the growth performance of broiler chickens. Formic acid may protect the chicks from the attack of pathogens (Pathak et al., 2017) and improve the nutrient digestibility and utilization (Hernández et al., 2006; Pathak et al., 2017). This may consequently promote the growth rate of chicks. It was shown in this study that formic acid supplementation increased feed intake in the IICC, which may also account for the improved weight gain of the IICC. In term of probiotic *S. cerevisiae*, Koc et al. (2010), Toader et al. (2018) and Mulatu et al. (2019) reported the beneficial effect of such probiotic yeast in increasing the weight gain of broiler chickens. The improvement of intestinal ecology, nutrient digestibility as well as the resistance of chicks to infections seemed to be responsible for the growth improvement in chickens (Akhavan-Salamat et al., 2011; Koc et al., 2010; Sugiharto, 2016; Mulatu et al., 2019). In this study, treatment with *S. cerevisiae* was also able to increase feed intake of the IICC. Accordingly, Akhavan-Salamat et al. (2011) demonstrated that feeding *S. cerevisiae* increased the digestibility and availability of Ca and P leading to the improved appetite of chicks. Interesting finding was observed in the present study, in which the blends of formic acid and *S. cerevisiae* resulted in better live body weight, FCR, feed cost per gain and IOFC of the IICC as compared to the individual administration of formic acid and *S. cerevisiae*, as well as control. Rodjan et al. (2018) formerly reported the better intestinal morphology and ecology in broiler chickens administrated with the blends of organic acids and probiotics, when compared with the individual administration of organic acids or probiotics. In our case, the synergistic works of formic acid and *S. cerevisiae* seemed to exert more superior effects on the growth performances in the IICC as compared to the single administration of formic acid or *S. cerevisiae*.

Our present study showed that the dietary supplementation of formic acid, *S. cerevisiae* or combination of both resulted in lower proventriculus relative weight of the IICC. This finding was in contrast to Nourmohammadi and Khosravinia (2015) reporting an increased proventriculus relative weight in broiler chickens with feeding citric acid, and Akyurek et al. (2011) showing no effect of organic acid blend on the proventriculus relative weight of modern broiler chickens. With regard to the effect of *S. cerevisiae*, our present finding was different from that of formerly revealed by Shareef and Al-Dabbagh (2009) and Koc et al. (2010) who found no effect of *S. cerevisiae* on the relative weight of proventriculus in broiler chickens. To date, there is no exact explanation for the above discrepancies, but the far greater body weight of the treated IICC (than control) before the slaughter may decrease the value of the proventriculus relative weight. Note that the body weight was used as the denominator for the determination of the organ relative weight of the IICC (Sugiharto et al., 2018). The same reason was also suggested for the lower duodenum relative weight of the treated IICC as compared to control.

Data in this current study showed the absent effect of dietary supplementation of formic acid, *S. cerevisiae* or the blends of both on the antioxidative status of the IICC, as reflected by the levels of SOD and MDA in the serum. Different from our finding, Aluwong et al. (2013) showed increased activities of the antioxidant enzymes in the serum of broiler chicks with the administration of *S. cerevisiae* in diets. In line with this, Abudabos and Al-Mufarrej (2014) reported that dietary supplementation with organic acids (FormaXOL) improved the total antioxidant activities of broiler chickens. Surai (2016) suggests that the levels of SOD and MDA in serum may be used as an indicator for stress-induced damage in poultry. During the rearing period, there were no notable factors that can induce stress in the IICC. For such reason, the serum activities of SOD and MDA may not be significantly activated in the IICC. The higher stress tolerance of the IICC (compared to modern broilers) may also explain the minimal change in serum activities of SOD and MDA in this present study. Indeed, Tirawattanawanich et al. (2011) reported that Thai indigenous crossbreed chickens exhibited lower stress levels compared to modern broiler strains. In this study, the antibody titer against NDV did not differ among the experimental groups. Indeed, the antibody titer against NDV was by far lower from the protected level ($\geq 3 \log_2$ GMT). Formerly, Rahman et al. (2017) noticed that antibody titers declined following the time post-vaccination. In the current work, NDV vaccination was carried out at day 4 and subsequently week 4, while the measurement was conducted at week 8. Such long time post-vaccination may therefore attributed to the weak titer of antibody against NDV at week 8.

Our present data showed that uric acid level was notably higher in the serum of the IICC supplemented with the blends of formic acid and *S. cerevisiae*. Simoyi et al. (2002) suggested that uric acid may serve as antioxidant agent, and thereby protects the chicks from oxidative stress. On this basis, the blends of formic acid and *S. cerevisiae* may be employed to improve the antioxidative status of the IICC. However, the latter inference should be interpreted with caution as the serum levels of SOD and MDA did not differ across the experimental groups in the present study.

In this study, treatment with the blends of formic acid and *S. cerevisiae* resulted in lower proportion of wings of the IICC. To date, the reason for the latter condition was not definitely known, as the corresponding data are scarce in the literature. Apart from the lower proportion of wings, dietary administration of formic acid, *S. cerevisiae* or the combination of both did not affect the eviscerated carcass of the IICC.

5. Conclusion

It can be concluded that feeding the blends of formic acid and *S. cerevisiae* improved growth performance and feed efficiency of the IICC. The combination of formic acid and *S. cerevisiae* was therefore essential to further increase the benefits of individual administration of formic acid or *S. cerevisiae* to the IICC in the post antibiotic-free era.

Declaration of competing interest

We state that we have no conflict of interest.

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