Original Research

Journal of Advanced Veterinary Research
(2024) Volume 14, Issue 1, 103-112

Efficiency of xylanase, emulsifier, and guanidinoacetic acid in restoring energy deficit in male broilers fed low metabolisable energy diets

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ARTICLE INFO

Recieved: 10 October 2023

Accepted: 30 November 2023

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Keywords:

Emulsifier
Gene expression
Guanidinoacetic acid
Gut morphology
Oxidative biomarker
Xylanase

ABSTRACT

The study aimed to examine the distinct impacts of incorporating xylanase (Xyl), emulsifier (EM), and guanidinoacetic acid (GAA) as dietary supplements in low metabolisable energy (ME) diets on performance, energy and protein efficiency ratios, oxidative biomarkers, gene expression, and gut morphology. Seven hundred one-day-old (Ross 308) male-broilers were assigned to 5 dietary treatments with 5 replicates of 28 birds each. The experimental group denoted as the positive control (PC) fed on diets in accordance with the breed recommendations. The negative control (NC) was subjected to a dietary intervention reducing the ME by 200 kcal/kg compared to the PC. The remaining experimental diets comprised NC diet that were supplemented with 0.01% Xyl, 0.03% EM and 0.06% GAA. Results showed that birds fed low-ME-diets increased their voluntary feed intake to meet their energy needs but was at the expense of their productive efficiency. Only NC+GAA partially restored broiler performance compared to PC. However, compared to the PC group Xyl, EM and GAA improved the energy and growth-related gene expression, oxidative biomarkers, and gut histomorphology ($p < 0.05$). The key features associated with Xyl and EM were growth-related genes and intestinal mucus, while GAA was associated with energy-related genes, oxidative biomarkers and jejunum-villi height and villus: crypt ratio. In conclusion, Xyl, EM and GAA supplementation to NC group were able to improve the health status of the birds. However, to improve the production efficiency, future research is needed to elucidate the effect of combined products in birds fed on such low ME diets.

Introduction

In the face of rising feed costs, poultry industry is seeking optimum ways to improve the production efficiency. One of the nutritional strategies is to feed broilers on low metabolisable energy diets. The latter can be cost-effective; however, this can negatively impact the growth and health of the birds. To mitigate these effects, various additives such as xylanase (Xyl), emulsifiers (EM) and guanidinoacetic acid (GAA) are used.

Xyl is an enzyme that breaks down xylan, the non-starch polysaccharides (NSP) in the plant cell walls. However, the type of feed is one among different factors that affects the efficiency of Xyl enzymes. For instance, corn-soy based diets contain higher level of insoluble NSP than soluble NSP. In such case, the reduction in gut viscosity is less important compared to the insoluble NSP. The latter, encapsulates starch and protein, acting as a direct barrier against the endogenous and exogenous enzymes. The addition of Xyl enzymes able to disrupt this encapsulating effect, substantially improves the access to starch and protein stored within the plant cell wall (Aftab, 2012; Nusairat and Wang, 2021). Additionally, the degradation of insoluble NSP by Xyl releases the Xylooligosaccharide (XOS) and arabinoxylooligosaccharide (AXOS), act as substrates for the fibrolytic bacteria in the cecum. These bacteria produce volatile fatty acids (VFA) and improve the intestinal health. The latter process takes time as the fibrolytic bacteria population needs to increase, so its effect is expected to be pronounced during the later stages of age (Dale *et al*., 2023). It has been reported that Xyl supplementation in low metabolisable energy diets can improve the productive performance (Williams *et al*., 2014; Craig *et al*., 2020), oxidative biomarkers (Bao and Choct, 2010; Zhang *et al*., 2018; Pirgozliev *et al*., 2021), and gut health (Luo *et al*., 2009; Khadem *et al*., 2016; Liu and Kim, 2017; Hosseini *et al*., 2018).

Another aspect to improve the production efficiency is using EM. It has been reported that EM increases fat digestibility, especially during early stage of growth, through formation of smaller emulsion droplets and faster micelle formation. Therefore, the fat surrounding other dietary components is reduced allowing faster availability of the substrates for the digestive enzymes (Saleh *et al*., 2020; Ghazalah *et al*., 2021; Oketch *et al*., 2022; Tenório *et al*., 2022). Moreover, age of the bird, and type and amount of fat are the most important factors that influence the effectiveness of EM (Tenório *et al*., 2022). Nevertheless, a recent study reported the effectiveness of EM in low metabolisable energy diets containing only fat from raw feed ingredients (Ghazalah *et al*., 2021). On the health aspect, the dietary supplementation of EM showed improvements in antioxidant parameter (Siyal *et al*., 2017; Saleh *et al*., 2020) and intestinal health (Ghazalah *et al*., 2021; Kubiś *et al*., 2022).

The digested nutrients (including the energy precursors), need to be further processed at cellular level. The efficiency of using adenosine-triphosphate (ATP), the ultimate product of energy metabolism, might be depending on the amount of creatine (Cre) at cellular level, which is crucial for shuttling the produced ATP from mitochondria to the cytoplasm to be either stored in the form of phosphocreatine (PCre) or to be used to support different physiological functions (Khalil *et al*., 2021a; Khalil *et al*., 2021b). Cre can be de novo synthesized in the animal's body, however in fast growing birds, skeletal muscles need enough oxygen and energy to be able to grow, which might be limiting factors in the nowadays improved broiler's genetics (Khalil *et al*., 2021b; Pirgozliev *et al*., 2022).

ISSN: 2090-6277/2090-6269/

Therefore, PCre may provide a readily available source of energy that may help in supporting faster growth (Khalil *et al*., 2021a; Khalil *et al*., 2021b). Guanidinoacetic acid (GAA), a precursor source of Cre, is the only form that is available for animal feed and has been proven to show energy sparing effect (Abudabos *et al*., 2014; Metwally *et al*., 2020; Pirgozliev *et al*., 2022), arginine sparing effect (Sharma *et al*., 2022) and maintaining gut integrity (Ahmadipour *et al*., 2018; Ren *et al*., 2018; Raei *et al*., 2020).

Modern broilers have become more efficient in energy utilization. According to (Aviagen, 2022) the metabolisable energy reduced by 25 kcal/kg feed in starter, 50 kcal/kg feed in grower and 100 kcal/kg feed in the finisher compared to (Aviagen, 2019). This necessitates a re-evaluation of aforementioned products, excluding the factor of genetic improvement. Therefore, this study aimed to evaluate the individual effects of each product supplementation in low metabolisable energy diets (-200 kcal/kg) on various aspects. These aspects include productive performance, energy and protein efficiency ratio, oxidative biomarkers, selected gene expression, and intestinal histomorphology of male broiler chicken. The study also aimed to describe the most important parameters of each observation. These parameters might be used as key factors in future research and application.

Materials and methods

Bird husbandry and experimental design

The handling and care of the birds was approved by the Institutional Animal Care and Use Committee at Vet. CU. IACUC (Vet CU 2009 2022465). The experiment was conducted in a semi-closed ventilation system in the Poultry Research Centre at the Faculty of Veterinary Medicine, Cairo University, Giza, Egypt. The house was subdivided into 25 equal floor pens of 2x 2 m dimension. Birds were reared in solid concrete floor, withwood shaving as a bedding material. A total of seven hundred one-day-old male chicks (Ross 308) were divided into 5 experimental groups with 5 replicates each (*n*= 28 birds/replicate). The first group was served as positive control group (PC) was fed on basal diets as recommended by breed manual (Aviagen, 2019). The second group was fed on reduced ME diets (200 kcal/kg) and served as a negative control (NC). Meanwhile, the third, fourth and fifth groups were fed on reduced energy diets either fortified with 0.01% xylanase (NC+Xyl) (Econase XT; Trichoderma reesei, 160,000 BXU/kg; AB Vista, UK) or 0.025% emulsifier (NC+EM) (Lysoforte extend lysolecithin, synthetic emulsifier and monoglycerides; Kemin, Belgium) or 0.06% guanidinoacetic acid (GAA) (Creamino, at least 96% GAA; AlzChem Trostberg GmbH, Trostberg, Germany), respectively. Feed and water were provided ad libitum. Birds were exposed to a continuous light program 24L:0D for the first 3 days of age. Day length was gradually reduced to 20L:4D until 35 days of age. For the first 3 days, the temperature was maintained at 32ºC, then gradually reduced by 0.50ºC/day until it declined to 24ºC at the end of experimental period. Throughout the experimental period the humidity was ranged between 55 and 60%. All birds were subjected to the recommended standard vaccination program against infectious diseases.

Diets

Corn-soy-based basal diets were formulated as per Aviagen guidelines (Aviagen, 2019) except the reduced energy groups (-200 kcal/kg). Starter, grower, and finisher diets were fed from 0-14, 14-26 and 26-35 days of age, respectively. All diets were mixed using a horizontal double ribbon mixer. Starter feeds were offered in crumbled form and then in pelleted (90ºC) form until the end of the experimental period (Table 1).

Sampling

On day 36 of age, five birds, representing the average pen weight, were selected from each pen in each group. Birds were slaughtered by severing the jugular vein. Liver, breast muscle and intestinal samples were collected for further analysis.

Measurements

Growth performance

Birds were initially weighed then body weight and feed intake (FI) were measured at the end of each feeding phase. Body weight gain (BWG) and feed conversion ratio (FCR) were calculated accordingly after adjusting mortalities. European poultry efficiency factor (EPEF) was determined by the following formula: EPEF = (average grams gained/day X % survival rate)/Feed Conversion X 10) according to Bawish *et al*. (2023).

Energy and protein efficiency ratio

Total protein intake (TPI) and total metabolisable energy intake (MEI) were recorded at the end of each feeding phase and cumulatively to determine the energy efficiency ratio (EER) and protein efficiency ratio (PER) using the following equations according to the methods described by Kamran *et al*. (2011).

MEI= FI x targets ME level of each phase/1000.

EER= WG/100 kcal energy intake.

TPI= FI x target crude protein of each phase/100.

PER= (WG /PI).

Oxidative biomarkers

The liver tissue (1 g) was homogenized in 9 mL of ice-cold PBS and then centrifuged at 2,500 xg for 15 min at 4°C, and the supernatant was collected to measure the levels of reduced glutathione (GSH) and total glutathione as well as glutathione peroxidase (GSH-Px) and superoxide dismutase activities (SOD) activities. The total glutathione and GSH levels (GSH+GSSG / GSH Assay Kit, No. ab239709), the total SOD (Superoxide Dismutase Activity Assay Kit, No. ab65354) and the total GSH-Px (No. ab102530) activities were determined using commercially available kits (Abcam, Cambridge Science Park, UK) in accordance with manufacturer's instructions.

Gene expression analysis

Breast muscle and liver samples were collected and stored at −80°C for subsequent analysis. Total RNA was isolated from different samples using a TRIzol reagent (Invitrogen, Carlsbad, CA, USA) as described in the manufacturer's instructions. The complementary DNA (cDNA) was synthesized using the Omniscript RT reagent kit (Qiagen) and real-time PCR was performed using the iQ SYBR Green Supermix (BIO-RAD, USA). All these operations were carried out according to the manufacturer's instructions. The mRNA levels of myogenin, myostatin, cystathionine-β-synthase (CBS), and insulin-like growth factor 1 (IGF-1) in the breast muscle as well as adenosine monophosphate-activated protein kinase (AMPK) in liver samples were examined. Primer sequences are shown in Table 2. PCR reactions took place on an Applied Biosystems QuantStudio 5 Real Time PCR system (Applied Biosystems, Foster City, CA, USA) with the following programs: 95°C for 30 s, 40 cycles at 95°C for 5 s and 60°C for 34 s, 95°C for 15 s, and a dissociation stage of 95°C for 15 s, 60°C for 1 min, 95°C for 15 s. Expression levels were normalized to β-actin and gene expression was calculated as $2^{-\Delta\Delta Ct}$ and expressed as relative fold change to control group, as described by Livak and Schmittgen (2001). In all real-time PCRs (RT-PCR) every single sample was measured in triplicate.

Intestinal histomorphology

Intestinal samples from duodenum, jejunum, and ileum were kept in neutral buffered formalin (10%) for fixation followed by routine pro-

cessing for hematoxylin and eosin staining (H&E) (Bancroft, 2013). Slides were examined using Leica DM 4B light microscope (Leica, Germany) and images were captured using Leica DMC 4500 digital camera (Leica, Germany). Intestinal villus height (from the tip of the villus to the villus-crypt junction) and crypt depth (from the base of crypt up to the villus-crypt junction) were measured using Image J software (1.45 s, National institute of health, USA); villus height to crypt depth ratio was then calculated (Abdelatty *et al*., 2021).

Statistical analysis

The data generated from productive performance, oxidative biomarkers, gene expression analysis, and intestinal histomorphology were subjected to one-way ANOVA using Minitab Statistical Software version 18. The positive control group (PC), as a reference group, compared with negative control groups by using protected Fisher's LSD test at $\alpha < 0.05$. Results shown in tables are means and their pooled standard error of means (SEM). Principal Component Analysis (PCA) of the studied parameters was analysed using XLSTAT software (Addinsoft, New York, NY, USA).

Results

Growth performance

Results (Tabe 3) showed that in the first two weeks, FI increased in the NC by 4% and in NC+Xyl group by 5.7% ($p < 0.05$). Meanwhile, FCR

Table 1. Ingredient (%) and chemical compositions of starter, grower, and finisher diets[1]

Ingredients	Starter (0-14 Day)		Grower (14-26 Day)		Finisher (26-35 Day)	
	PC	NC	PC	NC	PC	NC
Corn	52.42	51.86	57.23	61.51	62.04	66.4
Soybean meal (46% crude protein)	35	35	31.01	30.75	26.6	26.25
Corn gluten meal (60% crude protein)	3.88	3.7	3.46	3.13	2.52	2.23
Soya oil	3.75	1.93	4.55	0.86	5.34	1.63
Filler (Sand)	-	2.79	-	-	-	-
Dicalcium phosphate (18.2%)	1.39	1.39	1.17	1.16	1.05	1.04
Limestone	1.09	1.09	1.03	1.04	0.93	0.93
Soybeans full fat, toasted	0.85	0.63	-	-	-	-
L-Lysin HCL (78%)	0.32	0.32	0.3	0.31	0.28	0.29
Premix [2]	0.3	0.3	0.3	0.3	0.3	0.3
Salt (NaCl)	0.3	0.3	0.3	0.3	0.3	0.3
DL-Methionine (99%)	0.28	0.28	0.26	0.26	0.24	0.23
Mould inhibitor	0.15	0.15	0.15	0.15	0.15	0.15
Choline Chloride (60%)	0.1	0.1	0.1	0.09	0.11	0.11
L-Threonine (98.5%)	0.08	0.08	0.06	0.07	0.05	0.05
Sodium bicarbonate	0.07	0.07	0.07	0.07	0.08	0.08
Phytase[3]	0.01	0.01	0.01	0.01	0.01	0.01
Total	100	100	100	100	100	100
Chemical analysis (%)						
Dry matter	88.48	88.37	88.55	88.1	88.61	88.16
Crude protein	23.3	23.36	21.26	21.28	19	19
Fat	6.59	4.65	7.33	3.82	8.23	4.7
Metabolisable energy (kcal/kg)	3000	2800	3100	2900	3200	3000
Calcium	0.96	0.96	0.87	0.87	0.79	0.79
Available phosphorous	0.48	0.48	0.43	0.43	0.4	0.4
Potassium	0.97	0.97	0.89	0.89	0.8	0.81
Sodium	0.17	0.17	0.17	0.17	0.17	0.17
Chloride	0.21	0.21	0.21	0.21	0.21	0.22
Dietary electrolyte balance (mEq/kg)	263.42	263.48	240.83	242.33	219.6	220.63
Choline	1700	1700	1600	1600	1550	1550
Total lysine	1.44	1.44	1.3	1.3	1.16	1.16
Total methionine	0.65	0.65	0.6	0.6	0.55	0.54
Total methionine + cysteine	1.03	1.03	0.95	0.95	0.87	0.86
Total threonine	0.98	0.98	0.88	0.88	0.79	0.79
Total valine	1.11	1.11	1.02	1.02	0.91	0.91
Total arginine	1.51	1.51	1.36	1.36	1.21	1.2

[1]PC, positive control; NC, negative control; NC+Xyl, NC supplemented with 0.01% xylanase; NC+EM, NC supplemented with 0.025% emulsifier; NC+GAA, NC supplemented with 0.06% guanidinoacetic acid.
[2]Vitamins and mineral premix: vitamin A, 12,000,000 IU; vitamin D3, 2,200,000 IU; vitamin E, 10,000 mg; vitamin K3, 2,000 mg; vitamin B1, 1,000 mg; vitamin B2, 5,000 mg; vitamin B6, 1,500 mg; vitamin B12, 10 mg; niacin, 30,000 mg; pantothenic acid, 10000 mg; biotin, 50 mg; folic acid, 1000 mg; copper, 4000 mg; ferrous, 30,000 mg; manganese, 60,000 mg; zinc, 50,000 mg; iodine, 1000 mg; selenium, 100 mg.
[3]Quantum blue (AB-Vista) at 100 g/ton to provide 500 FTU and the following matrix value was considered (calcium, 1000%; phosphorus, 1000%; sodium, 350%; lysine, 170; methionine, 39; methionine + cysteine, 390; threonine, 330; arginine, 130; isoleucine 255; valine, 230).

increased by 8% in the NC+Xyl compared to the PC group ($p < 0.05$). From 14-26 days, WG decreased by 6% in both NC and NC+ Xyl and by 4% in the NC+GAA ($p < 0.05$). However, FCR increased by 10% and 6.5% in the NC and NC+Xyl group compared to the PC group, respectively ($p < 0.05$). From 26-35 days, only FI showed a tendency to be different among groups ($p = 0.064$). Overall results (1-35 days), compared to the PC, WG of NC groups was not significantly different from PC group ($p > 0.05$). Meanwhile, FI increased by 4.8% and 4.2% in the NC and NC+EM ($p < 0.05$), while FCR increased by 8.6%, 5.3% and 5.1% in the NC, NC+Xyl and NC+EM respectively ($p < 0.05$). FCR of the NC+GAA was comparable to PC group ($p > 0.05$). No significant difference in mortality % for all feeding phases and cumulatively ($p > 0.05$). EPPEF exhibited a trend towards statistically significance ($p = 0.07$), however, it is noteworthy that NC+GAA demonstrated a numerical similarity to the PC group.

Energy and protein efficiency ratios

Results indicated varying responses among groups with respect ME intake and TPI as well as their corresponding efficiency ratios as demonstrated in Table 4. In the starter phase, compared to PC group, the MEI of NC+EM and NC+GAA decreased by 3.7 and 7%, respectively ($p < 0.05$). Meanwhile, EER increased by 4 and 6% for the abovementioned parameters, respectively ($p < 0.05$). On the other hand, TPI of the NC and NC+Xyl group increased by 3.7 and 5.7%, and PER decreased by 4 and 7%, re-

Table 2. Nucleotide sequences of specific primers[1]

Gene symbol	Accession number	Orientation	Primer sequence (5´-> 3´)	Amplicon size (bp)
β-Actin	L08165	Forward	GAGAAATTGTGCGTGACATCA	152
		Reverse	CCTGAACCTCTCATTGCCA	
AMPK	NM_001039603	Forward	CGGCAGATAAACAGAAGCACGAG	148
		Reverse	CGATTCAGGATCTTCACTGCAAC	
CBS	XM_416752.3	Forward	CTGGGATCTTGAAACCTGGA	147
		Reverse	TCAGGACATCCACCTTCTCC	
Myogenin	D90157	Forward	AGCAGCCTCAACCAGCAGGA	149
		Reverse	TCT GCCTGGTCATCGCTCAG	
Myostatin	NM_001001461	Forward	TACCCGCTGACAGTGGATTTC	153
		Reverse	GCCTCTGGGATTTGCTTGG	
IGF-1	AY331392	Forward	CACCTAAATCTGCACGCT	140
		Reverse	CTTGTGGATGGCATGATCT	

[1]β-Actin: beta-actin; AMPK: adenosine monophosphate-activated protein kinase; CBS: cystathionine-β-synthase; IGF-1: insulin like growth factor 1

Table 3. Effects of xylanase, emulsifier and guanidinoacetic acid in low energy diets on growth performance up to 35 days of age[1,2]

Parameters[3]	PC	NC	NC+Xyl	NC+EM	NC+GAA	SEM[4]	p-Value
0-14 days							
WG (g)	492.6	491.86	481.6	494.46	486.86	6.38	0.62
FI (g)	575.09[a]	598.32[b]	608.02[b]	593.17[a]	572.87[a]	6.86	0.01
FCR (g: g)	1.168[a]	1.217[a]	1.264[b]	1.201[a]	1.178[a]	0.02	0.01
Mortality (%)	0.71	0.71	1.43	0.71	0	0.69	0.71
14-26 days							
WG (g)	1116.2[a]	1045.90[b]	1047.90[b]	1075.00[a]	1066.30[b]	15.27	0.03
FI (g)	1365.55	1411.7	1365.4	1386.05	1333.36	20.3	0.13
FCR (g: g)	1.224[a]	1.351[b]	1.303[b]	1.288[a]	1.253[a]	0.02	0.02
Mortality (%)	0.71	0.71	0	0.71	0	0.57	0.75
26-35 days							
WG (g)	825.5	811.4	823.18	844.4	825.34	30.89	0.96
FI (g)	1388.76	1476.9	1415.9	1490.74	1355.24	35.28	0.06
FCR (g: g)	1.69	1.83	1.73	1.78	1.64	0.05	0.13
Morality (%)	3.6	2.14	0.71	3.57	2.14	1.4	0.58
1-35 days							
WG (g)	2434.3	2349.16	2352.68	2413.86	2378.5	30.65	0.24
FI (g)	3329.40[a]	3486.86[b]	3389.32[a]	3469.96[b]	3261.47[a]	38.82	0.00
FCR (g: g)	1.368[a]	1.485[b]	1.441[b]	1.438[b]	1.37[a]	0.02	0.00
Mortality (%)	5	3.57	2.14	5	2.14	1.18	0.25
EPEF	483.98	436.6	457.02	455.92	485.22	12.94	0.07

[1]PC: diets contain standard metabolisable energy; NC: diets with reduced metabolisable energy by 200 kcal/kg; NC+Xyl: NC supplemented with xylanase (0.01%); NC+EM: NC supplemented with emulsifier (0.025%); NC+GAA: NC supplemented with guanidinoacetic acid (0.06%).
[2]Each mean represents 5 replicates with sample size = 15 birds/pen (75 birds/group).
[3]WG: weight gain; FI: feed intake; FCR: feed conversion ratio; EPEF: European poultry efficiency factor (average grams gained/day X % survival rate)/Feed Conversion X 10).
[a, b] means that not share a common superscript within a row differ significantly from PC as determined by Fisher's protected LSD ($p < 0.05$).
[4] SEM: standard error of means.

spectively ($p < 0.05$). In the grower phase, NC groups supplemented with Xyl, EM and GAA had lower MEI (-6.4, -5.0 and -8.7%, respectively) ($p < 0.05$). Meanwhile, PER in the NC group decreased by 9%, and in NC+Xyl by 6% compared to the PC group ($p < 0.05$). In the finisher phase, MEI and TPI showed a tendency to be different ($p = 0.070$ and 0.06, respectively). In the overall period, the MEI reduced by 4.7 and 8.3% in NC+Xyl and NC+GAA, respectively ($p < 0.05$). Meanwhile, NC+GAA had 6.5% increase in EER ($p < 0.05$). On the other hand, TPI of the NC and NC+EM increased by 4.7 and 4.1%, respectively ($p < 0.05$), while PER of NC and NC+Xyl and NC+EM reduced by 7.9, 5.4 and 4.8%, respectively, compared to PC group ($p < 0.05$).

Liver oxidative biomarkers

Results of the liver oxidative biomarkers are shown in Fig 1. Compared to PC, GSH activity decreased by 19% in NC but increased by 21 and 93% in NC+EM and NC+GAA, respectively ($p < 0.05$). on the other hand, T-GSH increased in NC+Xyl, NC+EM and NC+GAA by 16, 42 and 97%, respectively ($p < 0.05$). Moreover, NC+GAA showed a 47% increase in GSH-Px activity ($p < 0.05$). SOD activity in the NC decreased by 13% but increased by 21% in the NC+GAA ($p < 0.05$).

Relative PCR gene expression

Results concerning mRNA gene expression are shown in Fig. 2. Breast muscle myogenin, myostatin, IGF and CBS of NC group were not different compared to the PC group ($p > 0.05$). Meanwhile, NC+Xyl, NC+EM and NC+GAA had significant fold changes on aforementioned parameters. Compared to the PC group, a significant increase in gene expression for myogenin by (11.87-, 10.49- and 6.22-fold), myostatin by (7.56-, 7.74- and 5.05-fold), IGF by (8.42, 9.65 and 10.95) and CBS by (1.96-, 2.11- and 10.28-fold) in NC+Xyl, NC+EM and NC+ GAA, respectively ($p < 0.05$). Meanwhile, hepatic AMPK gene expression in the NC, NC+Xyl, NC+EM and NC+GAA increased by 2.77-, 10.82-, 2.79- and 12.23-fold, respectively ($p < 0.05$).

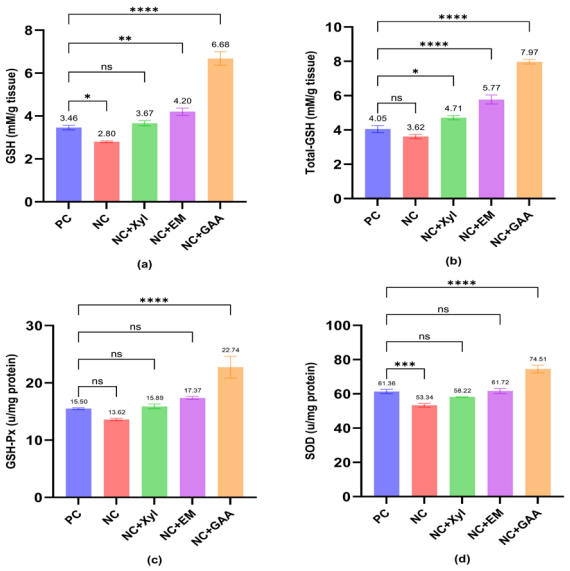

Fig. 1. Levels of (a) GSH: reduced glutathione, (b) T-GSH: total glutathione, (c) GSH-Px: glutathione peroxidase, (d) SOD: superoxide dismutase in the liver from PC, NC, NC+Xyl, NC+EM, and NC+GAA groups. Data are expressed as mean ± SE of five birds per treatment group and were analyzed using one-way ANOVA followed by Fisher's protected LSD post hoc test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ significance when compared to PC group. ns = not significant).

Table 4. Effects of xylanase, emulsifier and guanidinoacetic acid in low energy diets on total etabolisable energy intake, energy efficiency ratio, total protein intake and protein efficiency ratio[1,2]

Parameters[3]	PC	NC	NC+Xyl	NC+EM	NC+GAA	SEM[4]	p-Value
1-14 days							
Total ME intake (kcal)	1725.28[a]	1675.31[a]	1702.45[a]	1660.87[b]	1604.05[b]	19.5	0.00
EER (gain/100 kcal ME)	28.56[a]	29.3 7[a]	28.34[a]	29.78[b]	30.36[b]	0.41	0.01
Total protein Intake (g/bird)	134.00[a]	139.41[b]	141.67[b]	138.21[a]	133.48[a]	1.6	0.01
PER (g: g)	3.68[a]	3.53[b]	3.41[b]	3.58[a]	3.65[a]	0.05	0.01
14-26 days							
Total ME intake (kcal)	4233.20[a]	4093.84[a]	3959.79[b]	4019.54[b]	3866.74[b]	59.29	0.00
EER (gain/100 kcal ME)	26.36	25.58	26.47	26.77	27.63	0.52	0.13
Total protein Intake (g/bird)	290.32	300.4	290.57	294.95	283.74	4.32	0.13
PER (g: g)	3.84[a]	3.49[b]	3.61[b]	3.65[a]	3.77[a]	0.07	0.02
26-35 days							
Total ME intake (kcal)	4444.03	4430.59	4247.59	4472.22	4065.71	108.43	0.07
EER (gain/100 kcal ME)	18.6	18.33	19.37	18.84	20.32	0.54	0.11
Total protein Intake (g/bird)	263.86	280.6	269.01	283.24	257.49	6.7	0.06
PER (g: g)	3.13	2.89	3.06	2.97	3.21	0.09	0.13
1-35 days							
Total ME intake (kcal)	10402.50[a]	10199.74[a]	9910.83[b]	10153.63[a]	9536.49[b]	117	0.00
EER (gain/100 kcal ME)	23.41[a]	23.04[a]	23.74[a]	23.78[a]	24.95[b]	0.28	0.00
Total protein Intake (g/bird)	688.18[a]	720.42[b]	701.25[a]	716.4[b]	674.71[a]	7.71	0.00
PER (g: g)	3.54[a]	3.26[b]	3.35[b]	3.37[b]	3.53[a]	0.04	0.00

[1]PC: diets contain standard metabolisable energy; NC: diets with reduced metabolisable energy by 200 kcal/kg; NC+Xyl: NC supplemented with xylanase (0.01%); NC+EM: NC supplemented with emulsifier (0.025%); NC+GAA: NC supplemented with guanidinoacetic acid (0.06%).
[2]Each mean represents 5 replicates with sample size = 15 birds/pen (75 birds/group).
[3]Total ME intake: feed intake x targets ME level of each phase/1000; EER: energy efficiency ratio (weight gain/100 kcal energy intake; Total protein intake: Feed intake x target crude protein of each phase/100; PER: protein efficiency ratio (weight gain/protein intake).
[a, b] means that not share a common superscript within a row differ significantly from PC as determined by Fisher's protected LSD ($p < 0.05$).
[4]SEM: standard error of means.

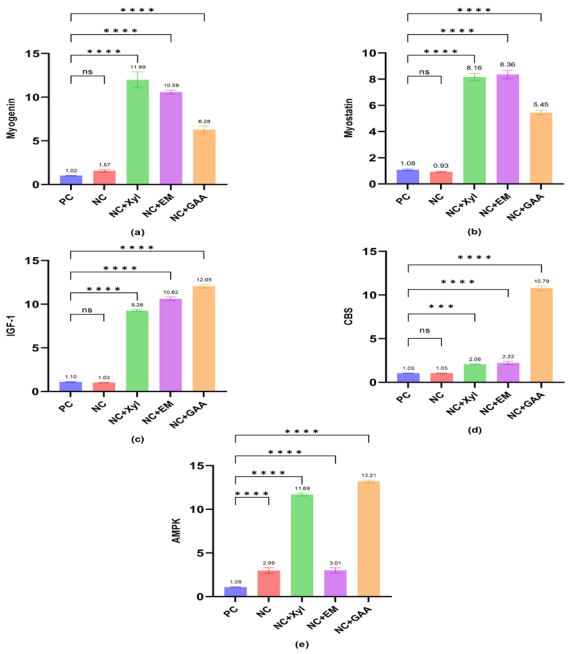

Fig. 2. Relative PCR gene expression of (a) myogenin, (b) myostatin, (c) IGF-1: insulin like growth factor-1, (d) CBS: cystathionine-β-synthase in the breast muscle, and (e) AMPK: adenosine monophosphate-activated protein kinase in the liver samples from PC, NC, NC+Xyl, NC+EM and NC+GAA groups. Data are expressed as mean ± SE of five birds per treatment group and were analyzed using one-way ANOVA followed by Fisher's protected LSD post hoc test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ significance when compared to PC group. ns = not significant).

Intestinal histomorphology

Results regarding intestinal histomorphology are shown in (Table 5). Histopathological examination of the intestinal segments of different groups revealed normal histological structure of the intestinal villi and crypts with intact submucosa and absence of any abnormalities Fig. 3(a). Examination of mucous secretion revealed hyperplasia of goblet cells in duodenum, jejunum, and ileum, respectively in all groups Fig. 3(b).

Duodenum histomorphology showed that the villus height of NC group was significantly decreased by 10% compared to the PC group ($p < 0.05$.). Crypt depth of NC+Xyl decreased by 24% however, villus: crypt ratio increased by 36% ($p < 0.05$). Mucus area percentage increased by 86, 71, 73 and 39% in NC group, NC+Xyl, NC+EM and NC+GAA, respectively compared to PC group ($p < 0.05$).

In the jejunum segment, the villus heights of NC+Xyl, NC+EM and NC+GAA increased by 23.93, 28.94 and 42.40%, respectively ($p < 0.05$). Crypt depth of NC+EM and NC+GAA increased by 11.91 and 13.90%, respectively ($p < 0.05$). Villus: crypt ratio of NC+Xyl, NC+EM and NC+GAA increased by 16.72, 17.44 and 25.73%, respectively ($p < 0.05$). Mucus area percentage increased by 35, 36 and 29% in NC, NC+Xyl and NC+EM compared to PC, respectively ($p < 0.05$).

In the ileum segment, the villus height of the NC+EM increased by 8.29%, whilst NC+GAA decreased by 21.96% ($p < 0.05$). Villus: crypt ratio of the NC+GAA decreased by 20% ($p < 0.05$). Mucus area percentage increased by 20, 22, 59 and 18% in the NC group, NC+Xyl, NC+EM and NC+GAA compared to the PC group, respectively ($p < 0.05$).

Principal component analysis

The principal component analysis (PCA) biplot of the MEI, PI and their efficiency ratios, gene expression, and oxidative biomarkers is shown in Fig. 4(a). PC1 and PC2 axes explained 91.36% of the total variance. The first PC (68.35% of the total variance) was positively loaded by IGF-1, CBS, AMPK, EER, GSH, T-GSH, GSH-Px and SOD but negatively loaded by MEI and PI. The second PC (23.02% of total variance) was positively loaded by myogenin and myostatin but negatively loaded by PER. In the biplot, NC+GAA was on the same side of IGF-1, CBS, AMPK, EER, GSH, T-GSH, GSH-Px and SOD. On the other hand, NC was on the opposite side of the aforementioned parameters but was more associated with MEI and PI. Meanwhile, NC+Xyl and NC+EM were positively associated with myogenin and myostatin but negatively associated with PER. However, PC was negatively associated with myogenin and myostatin but positively associated with PER.

The PCA biplot of the MEI, PI, intestinal histomorphology and mucus is shown in Fig. 4(b). PC1 and PC2 axes explained 72.10% of the total variance. The first PC (41.74% of the total variance) was positively loaded by I-V:C, J-mucus, I-villi, I-crypt, MEI, and PI but negatively loaded by J-V:C and J-villi. The second PC (30.36%) was positively loaded by D-mucus, I-mucus and J-crypt but negatively loaded by D-crypt. In the biplot, NC group was positively associated with I-V:C, J-mucus, I-villi, I-crypt, MEI, and PI but was negatively associated with J-V:C and J-villi. On the oppo-

Table 5. Effects of xylanase, emulsifier and guanidinoacetic acid in low energy diets on intestinal histomorphology[1,2]

Parameters	PC	NC	NC+Xyl	NC+EM	NC+GAA	SEM[3]	p-Value
Duodenum							
Villus height (µm)	1786.69[a]	1604.92[b]	1809.75[a]	1831.19[a]	1749.78[a]	23.92	<0.0001
Crypt depth (µm)	203.80[a]	200.17[a]	154.03[b]	199.19[a]	205.36[a]	5.8	<0.0001
Villus: crypt ratio	9.17[a]	8.24[a]	12.51[b]	9.65[a]	8.88[a]	0.34	<0.0001
Mucus (area, %)	2.33[a]	4.33[b]	3.99[b]	4.02[b]	3.24[b]	0.29	<0.0001
Jejunum							
Villus height (µm)	786.93[a]	818.45[a]	975.23[b]	1014.68[b]	1120.60[b]	19.68	<0.0001
Crypt depth (µm)	118.26[a]	116.90[a]	126.77[a]	132.34[b]	134.70[b]	3.85	0.00
Villus: crypt ratio	6.88[a]	7.20[a]	8.03[b]	8.08[b]	8.65[b]	0.26	<0.0001
Mucus (area, %)	8.20[a]	11.07[b]	13.40[b]	10.60[b]	6.41[a]	0.69	<0.0001
Ileum							
Villus height (µm)	767.30[a]	720.94[a]	751.22[a]	830.90[b]	598.76[b]	18.28	<0.00001
Crypt depth (µm)	124.04	126.49	126.11	130.51	119.3	4.18	0.43
Villus: crypt ratio	6.53[a]	6.21[a]	6.15[a]	6.75[a]	5.22[b]	0.26	0.00
Mucus (area, %)	16.50[a]	19.38[b]	20.11[b]	26.23[b]	19.41[b]	0.96	<0.0001

[1]PC: diets contain standard metabolisable energy; NC: diets with reduced metabolisable energy by 200 kcal/kg; NC+Xyl: NC supplemented with xylanase (0.01%); NC+EM: NC supplemented with emulsifier (0.025%); NC+GAA: NC supplemented with guanidinoacetic acid (0.06%).
[2]Each mean represents 50 slide section from 5 birds/treatment.
[a, b] means that not share a common superscript within a row differ significantly from PC as determined by Fisher's protected LSD ($p < 0.05$).
[3]SEM: standard error of means.

site side, NC+GAA was positively associated with J-V:C and J-villi but negatively associated with I-V:C, J-mucus, I-villi, I-crypt, MEI, and PI. On the other hand, NC-Xyl and NC-EM were positively associated with D-mucus, I-mucus, J-crypt but negatively associated with D-crypt. On the opposite side, PC was negatively associated with D-mucus, I-mucus, J-crypt but positively associated with D-crypt.

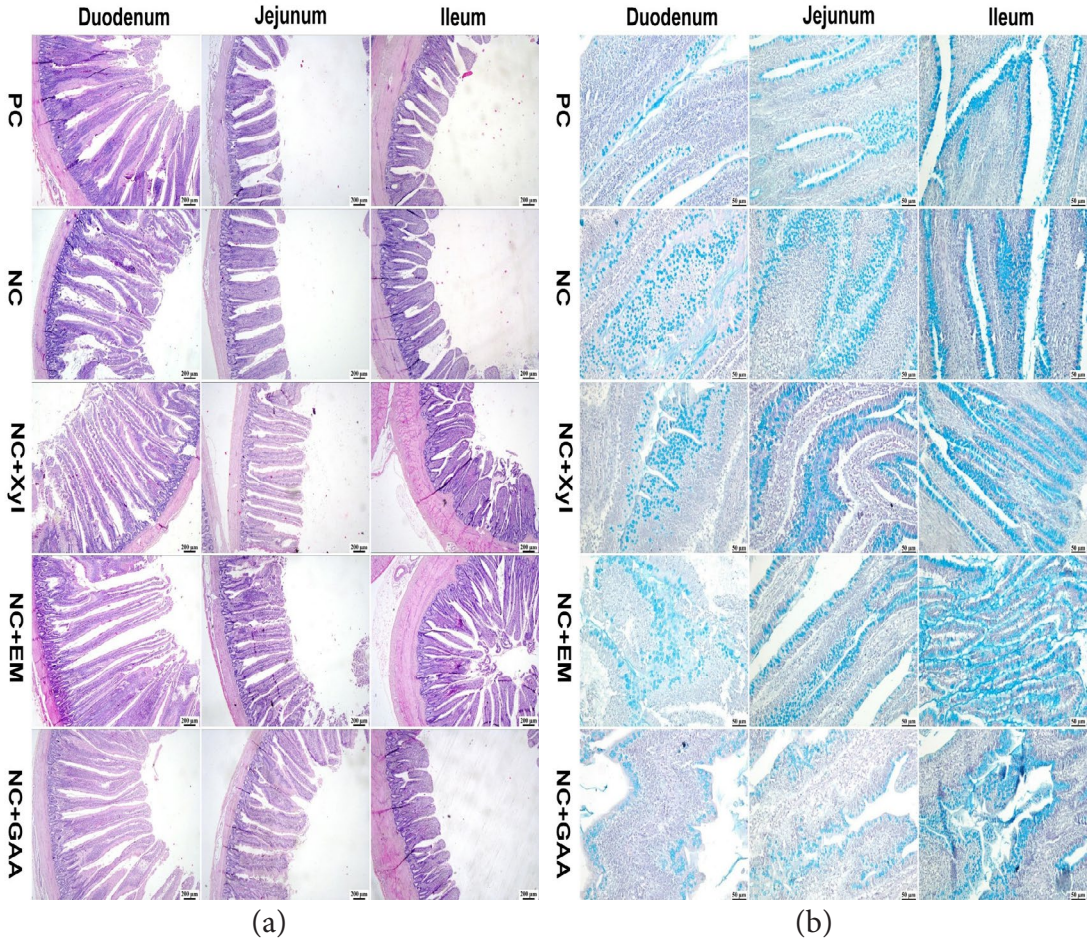

Fig. 3. (a) shows intestinal histomorphology of broiler chickens of different groups. Normal histological structure of different intestinal regions (H&E stain), intestinal mucosa was pleated into folds (villi) that appeared very long and thin in the duodenum, a little broad and long in the jejunum and broader and short in the ileum. Scale bar, 200 µm. (b) Goblet cells stained blue with alcian blue stain in the intestinal mucosa. A marked increase of mucus secretion was observed in the ileum segment of different groups especially in NC+EM group. Scale bar, 50 µm.

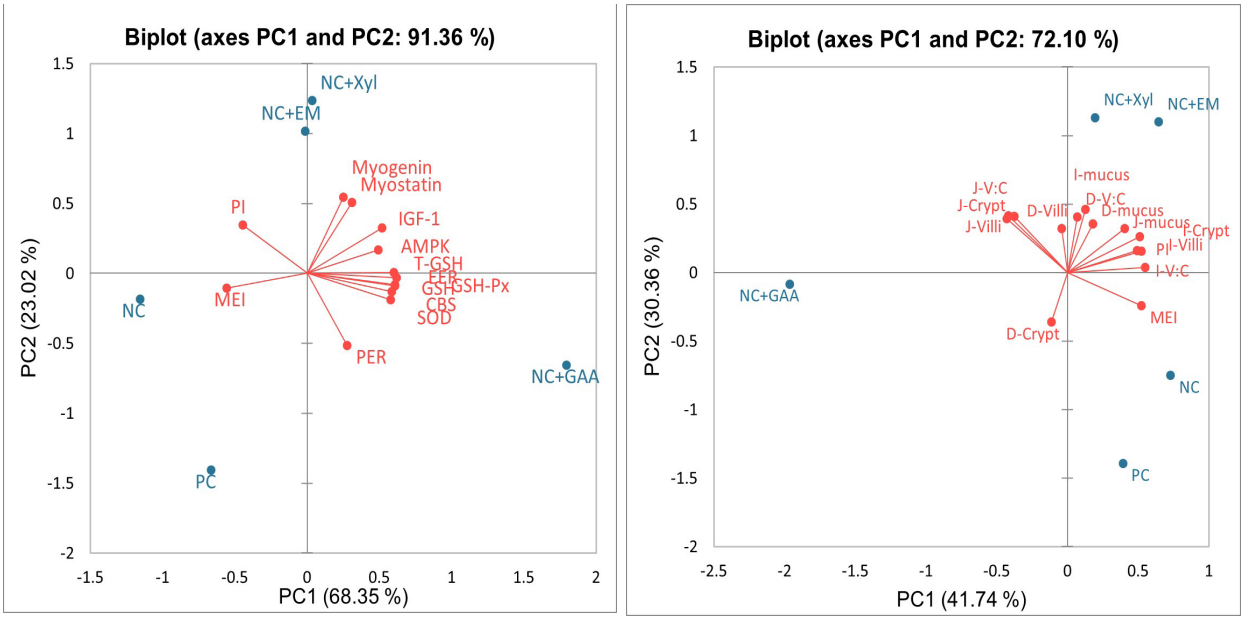

Fig. 4. Principal component analysis biplot of the observations represented by blue dots and variables represented by red dots. The abbreviation for the observations in (a) and (b) is as following: PC: positive control; NC: negative control; NC+Xyl: negative control supplemented with xylanase; NC+EM: negative control supplemented with emulsifier; NC+GAA: negative control supplemented with guanidinoacetic acid. The abbreviation for variables is as following: (a) MEI: metabolisable energy intake; PI: protein intake; EER: energy efficiency ratio; PER: protein efficiency ratio; IGF-1: insulin-like growth factor-1; AMPK: adenosine monophosphate-activated protein kinase; T-GSH: total glutathione; GSH: reduced glutathione; GSH-Px: glutathione peroxidase; CBS: cystathionine-β-synthase; (b) D-villi: duodenum villi; D-Crypt: duodenum crypt; D-V:C: duodenum villus: crypt ratio; J-villi: jejunum villi; J-crypt: jejunum crypt; J-V:C: jejunum villus: crypt ratio; I-villi: ileum villi; I-crypt: ileum crypt; I-V:C: ileum villus: crypt ratio; D-mucus: duodenum mucus; J-mucus: jejunum mucus; I-mucus: ileum mucus.

Discussion

Birds eat to meet energy demands for maintenance and growth. On low ME diets, energy is firstly used for maintenance, then growth. Therefore, growth may be affected due to energy deficit (Hosseini *et al*., 2018). In accordance with our findings, previous studies revealed that lowering ME in the diet negatively affected the FCR (Castro *et al*., 2019; Bromfield *et al*., 2021; Ebbing *et al*., 2022). However, FI and BWG were controversial among different studies (Chang *et al*., 2015; Castro *et al*., 2019; Bromfield *et al*., 2021; Ebbing *et al*., 2022) possibly due to different ages, gender, management conditions and the physiological adaptation of different breeds (Dozier and Gehring, 2014).

Many research activities confirmed the ability of Xyl to improve the productive performance of broiler chickens fed low ME diets (Williams *et al*., 2014; Tang *et al*., 2017; Selim *et al*., 2018; Craig *et al*., 2020). However, when energy reduced ≥ 200 kcal/kg, the birds failed to reach the breed performance objectives (Chang *et al*., 2015; Tang *et al*., 2017; Ebbing *et al*., 2022). The latter is consistent with our results.

On the other hand, EM supplementation showed to be effective in both diets with different fat sources and levels (Jansen *et al*., 2015; Wealleans *et al*., 2020) and those with low ME level (-100 kcal/kg ME) (Zhao and Kim, 2017; Chen *et al*., 2019). However, according to Boontiam *et al*. (2019) 0.05% EM supplementation to low ME diet (-150 kcal/kg) was unable to improve feed efficiency, which may have been due to the relatively low dietary fat content. Our results were not in agreement with Ghazalah *et al*. (2021) who reported the effectiveness of EM in the absence of added fat in low ME diet.

Several research activities confirmed that GAA supplementation could spare from 50 to 100 kcal/kg ME in broilers without compromising the performance level (Abudabos *et al*., 2014; Metwally *et al*., 2020; Ceylan *et al*., 2021; Pirgozliev *et al*., 2022). Our result was more or less similar to Mousavi *et al*. (2013); Fosoul *et al*. (2018) who reported that adding 0.06% GAA to low ME diet (-150 kcal/kg) maintained the ADFI and FCR compared to the standard energy diet.

Interestingly, the weight gain across all NC groups was not statistically different. This lack of statistical sensitivity might be due to the wide variation within each group. However, this variation could also illustrate the physiological dynamics of the birds, as they may prioritize using energy for maintenance before growth when under low ME diets.

In our study, calculating the total MEI and TPI is of interest to better understand the physiological dynamics in response to low ME diet. Similar to our findings, Maliwan *et al*. (2022) reported that the EER of groups fed on low energy diets were not statistically different compared to standard ME group, but TPI was increased at slaughter age, significantly.

Our results were more or less in agreement with Oyeagu *et al*. (2019) who confirmed that Xyl supplementation reduced the MEI and increased the EER ($p < 0.05$). However, the TPI decreased and PER increased compared to un-supplemented group ($p < 0.05$). Meanwhile, The NC+Xyl group in our study maintained the TPI, but PER was compromised compared to PC group ($p < 0.05$).

During the starter and grower periods, NC+EM decreased the MEI ($p < 0.05$) and improved the EER during the starter phase ($p < 0.05$) compared to the PC group, which may demonstrate the importance of EM supplementation during the early stage. On the hand, the cumulative PI increased ($p < 0.05$) resulted in a decrease in PER ($p < 0.05$). To the best of our knowledge, there is limited or no data available to validate our findings. Therefore, further studies are required for more data accuracy.

Interestingly, the NC+GAA group lowered the overall MEI and improved the EER ($p < 0.05$), while maintaining the TPI and the PER compared to the PC group. Our findings may highlight the role of Cre-PCre shuttle system in maintaining the intracellular energy homeostasis that resulted in achieving the perfect balance between the energy utilization and muscle accretion under low ME diet. No similar data were found to be compared to our study. Further studies are needed to elucidate the effect of GAA in low ME on EER and PER for more data accuracy.

The NC group in our study showed oxidative stress as indicated by the reduction in hepatic GSH and SOD activities compared to the PC group ($p < 0.05$). The lower antioxidant capacity in the NC group could be related to the high TPI and low PER. The excess PI might be catabolized and directed to form the glucogenic and ketogenic amino acids for energy production, which may result in amino acids imbalance and formation of nitrogen waste. This may lead to limitations in the antioxidant precursors, which would reduce the antioxidant capacity (Parmeggiani and Vargas, 2018).

In our study NC+Xyl group improved the T-GSH ($p < 0.05$), while GSH, GSH-Px and SOD were maintained compared to PC group. Zhang *et al*. (2018) found that dietary Xyl supplementation improved the total antioxidant capacity, SOD, GSH-Px and MDA in the serum ($p < 0.05$). However, Pirgozliev *et al*. (2021) found that GSH-Px activity in the serum was not affected by Xyl supplementation, which is consistent with our findings. The mechanism by which Xyl may improve the antioxidant status is not fully understood. However, it had been assumed that Xyl may shift the microbial populations toward beneficial micro-organism through NSP degradation to OS and release more phenolics compounds that act as antioxidant. Moreover, it had been suggested that dietary fibre anti-oxidants exist in insoluble NSP (Bao and Choct, 2010).

NC+EM group improved the antioxidant status under current experimental condition. The improvement in antioxidant status was demonstrated by several studies.

Saleh *et al*. (2020) reported that EM improved hepatic lipid peroxidation. Moreover, Siyal *et al*. (2017) revealed that 0.1% soy lecithin supplementation increased the hepatic catalase, SOD, and total antioxidant capacity, which resulted in a reduction of hepatic MDA ($p < 0.05$). Our results indicated that NC+EM may improve the fatty acids, fat-soluble vitamins, carotenoids, and cell membrane permeability, which could maximize the availability of α-tocopherol. This could lead to an improvement in antioxidant status (Saleh *et al*., 2020). Moreover, phospholipids can contribute a hydrogen atom from an amino group, which may trigger oxidized phenolic compounds that act as true antioxidants (Siyal *et al*., 2017).

The improvement in the antioxidant status in NC+GAA group in our study was supported by many previous research (Khalil *et al*., 2021a; Zhao *et al*., 2021; Al-Shammari, 2023). It is believed that the role of Cre-PCre shuttle system may offer better regulation of mitochondrial activity, hence lowering the mitochondrial dependent-ROS production (Liu *et al*., 2002; Glancy *et al*., 2008). It has been demonstrated that GAA can mitigate the negative impact of T3-hormone-induced stress on mitochondria and improve the antioxidant status (Khalil *et al*., 2021a).

In accordance with our findings, previous research has confirmed the activation of AMPK pathway in response to low ME diet (Kim *et al*., 2016; Hu *et al*., 2019). Moreover, it was reported that breast muscle myostatin was not influenced by low ME diets (Yang *et al*., 2009; Saxena *et al*., 2020). Additionally, Saxena *et al*. (2020) revealed that the IGF-1 expression decreased in groups fed on low ME diets regardless the crude protein level compared with high energy diets ($p < 0.05$). Another study showed that the low ME diet did not affect the IGF-1 and myogenin, but myostatin was downregulated compared to standard diet ($p < 0.05$) (El Sayed *et al*., 2017). It is worth noting that myostatin not only suppresses the skeletal muscle growth but also regulates the glucose metabolism through the activated AMPK signal pathway (Chen *et al*., 2010). The fold increase in liver AMPK expression in the NC group was insufficient to upregulate myostatin gene expression under the current experimental conditions, which may result in poor glucose uptake to produce ATP required for growth. There is not data available regarding the effect of low ME diet on CBS gene expression.

NC+Xyl, NC+EM and NC+GAA groups showed upregulations of the IGF-1, myogenin, myostatin, AMPK, and CBS expression compared to PC group ($p < 0.05$). In consistent to our results, previous research works confirmed that dietary Xyl supplementation significantly increased IGF-1 (Gao *et al*., 2007, 2008). Meanwhile, Zhang *et al*. (2022) reported an increase in relative mRNA expression of myogenin and IGF-1 in EM treated group ($p < 0.05$). Additionally, it was reported that GAA supplementation upregulated the mRNA gene expression of myogenin and IGF-1 (Metwally *et al*., 2015; Farshidfar *et al*., 2017; Li *et al*., 2022). To the best of our knowledge, not enough studies discussed the effect of Xyl, EM and GAA on myostatin, AMPK and CBS gene expression. More research works are required to validate our findings.

Results showed that the NC+Xyl group improved the histomorphology of the small intestine, which is in accordance with previous studies that confirmed Xyl's beneficial effect (Luo *et al*., 2009; Khadem *et al*., 2016; Liu and Kim, 2017; Hosseini *et al*., 2018). Xyl may improve intestinal histomorphology by producing OS and XOS, which serve as available substrates for the fibrolytic bacteria that produce volatile fatty acids (Bautil *et al*., 2021).

NC+EM showed improvements in the jejunum and ileum villi heights, and the villus: crypt ratio compared to PC group ($p < 0.05$). Supporting to our results,

Nascimento *et al*. (2022); Tenório *et al*. (2022) reported that supplementation of EM did not improve the duodenum height but improve the villus: crypt ratio. However, other studies reported improvements in jejunal (Ghazalah *et al*., 2021) and ileal villi height (Oketch *et al*., 2022). The improvements in the intestinal histomorphology might be related to the enhancement of fat digestion and the incorporation of lysolecithin in the epithelial cell walls (Saleh *et al*., 2020; Wealleans *et al*., 2020; Ghazalah *et al*., 2021).

In our study, NC+GAA maintained the duodenum and improved the jejunum histomorphology. However, the villus height and villus: crypt ratio of the ileum were lower than the PC group ($p < 0.05$). Previous studies reported that dietary GAA supplementation improved the duodenum (Ahmadipour *et al*., 2018; Ren *et al*., 2018; Amiri *et al*., 2019), jejunum (Ah-

madipour *et al*., 2018; Ren *et al*., 2018). and ileum histomorphology (Ahmadipour *et al*., 2018; Ren *et al*., 2018; Raei *et al*., 2020). The improvement in the intestinal histomorphology could be referred to the arginine sparing effect of GAA, which has been reported in several studies (Murakami *et al*., 2012; Ghamari Monavvar *et al*., 2020; Sharma *et al*., 2022).

Regarding the intestinal mucus area percentage, the NC, NC+Xyl, NC+EM and NC+GAA had greater mucus compared to PC group. Nevertheless, NC+GAA reduced the jejunum mucus ($p < 0.05$). The results were surprising because there was no histopathological lesion found among the experimental groups. Therefore, it might be related to the low ME diets or the low dietary fat or changes in the microbiome. Limited or no data are available concerning mucus secretion in low energy diets. Therefore, further research is warranted to investigate the relationship between mucus secretion, low fat diet and changes in the microbiome.

Several of our recorded variables may not be independent. Therefore, PCA was used to describe the relationships among variables and to identify the most important parameters contributing to the reduced ME in the diet.

In our study, PCA showed that the low MEI and high EER increased IGF-1, CBS, and the AMPK gene expression. The latter requires CBS domain that bind the adenine nucleotides to the γ-subunit of AMPK in order to stimulate its activity in response to the high AMP and to lesser extent ADP (Herzig and Shaw, 2018). The activated AMPK may increase the glycolysis and reduce the glycogen storage in the liver to increase the blood glucose level. The latter may explain the upregulation of IGF-1 to increase the glucose uptake (Clemmons, 2004; Kim *et al*., 2016).

Interestingly, PCA showed that the EER were positively correlated with the oxidative biomarkers. The latter was strongly associated with CBS gene expression. The upregulation of CBS gene expression may increase cystine production which, is a precursor of the powerful antioxidant GSH and taurine (Ables, 2021). In addition to the previous role, cystine is a glucogenic amino acids that can be converted to pyruvate to during glucose limitation (D'Mello, 2003).

On the other hand, myogenin and myostatin were negatively associated with PER. The increased PI might be used in the energy metabolism through the glucogenic and ketogenic amino acids, leaving limited amino acids building blocks for protein synthesis (D'Mello, 2003).

Regarding the intestinal histomorphology, the increased MEI and PI were associated with increased jejunum-mucus, ileum-villi, ileum-crypt, and ileum-villus: crypt ratio but were negatively associated with jejunum-villi and jejunum-villus: crypt ratio. It is well known that mucus plays a key role in the intestinal barrier against the pathogenic bacterial (Svihus, 2014). In our study, no pathological picture was noticed among groups. Therefore, it might be speculated that the increased mucus might be related to the changes in the microbiome that resulted in low jejunum-villi height and jejunum-villus crypt ratio. Nevertheless, the improvements in ileum-villi height, ileum-crypt, and ileum-villus: crypt ratio might be a compensatory mechanism to maximize the feed utilization (Svihus, 2014).

NC supplemented with Xyl, EM and GAA improved the gene expression, oxidative biomarkers and intestinal histomorphology with different magnitude compared to PC group. Since they have different mode of actions, PCA was performed to visualize the potential of reducing the number of variables that needed to describe the physiological dynamics and highlighting the most important parameters that related to each observation, which could be used as key factors.

NC supplemented with Xyl and EM were positively associated with myogenin and myostatin but negatively associated with PER. Meanwhile, GAA was strongly associated with AMPK, CBS, IGF-1, EER and oxidative biomarkers and negatively associated with MEI, PI.

Regarding the intestinal histomorphology, NC supplemented with Xyl and EM were positively associated with duodenum-mucus, ileum-mucus and jejunum-crypt but negatively associated with duodenum-crypt. On the other hand, GAA was positively associated with jejunum-villi and jejunum-villus: crypt ratio but negatively associated with jejunum-mucus, ileum-villi, ileum-crypt, and ileum-villus: crypt ratio.

Conclusion

Birds fed low ME diets are able to meet their energy demands by increasing their voluntary FI, but this came at the expense of productivity and feed efficiency. The NC supplemented with either Xyl or EM is unable to restore broiler performance to the level of the PC group at such a low ME level. However, the NC+GAA group partially restore broiler performance compared to the PC group. The NC supplemented with Xyl, EM, and GAA increased energy and growth-related mRNA gene expression, oxidative biomarkers, and histomorphology. The key features associated with Xyl and EM were the growth-related genes and the intestinal mucus surface area percentage. In contrast, the key features associated with NC supplemented with GAA were AMPK, CBS, IGF-1, EER, oxidative biomarkers, and the jejunum-villi height and villus: crypt ratio (Fig. 4). Future research is needed to elucidate the effect of combined products on birds fed low ME diets.

Acknowledgments

The authors sincerely thank all individuals involved in this project from the Poultry Research Centre at Vet. Med. Cairo University for their hard work and dedication. Additionally, the authors thank Alzchem Trostberg GmbH for their advice.

Conflict of interest

No potential conflict of interest was reported by the authors.

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