Multi-carbohydrase effects on energy utilization depend on soluble non-starch polysaccharides-to-total non-starch polysaccharides in broiler diets

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ABSTRACT Non-starch polysaccharides (**NSP**), especially in water-soluble form, are a common anti-nutritional factor in cereal-based poultry diets. Consequently, carbohydrases are applied to diets to combat the negative effects of NSP on bird performance and health, particularly when feeding viscous grains. This study investigated the effect of supplementing multi-carbohydrases (**MC**) to broiler diets containing either low (**LS**) or high (**HS**) soluble NSP (**sNSP**) to total NSP (**tNSP**) ratios on energy partitioning, nitrogen (**N**) balance, and performance. A 2 × 2 factorial arrangement of treatments (MC, no or yes; sNSP/tNSP, LS vs. HS) was applied, resulting in 4 dietary treatments, each replicated 8 times. These treatments were fed to Ross 308 broilers in closed-circuit indirect calorimetry chambers, with 2 birds (a male and a female) per replicate chamber (n = 64). The results showed that MC addition increased AME, net energy (**NE**), and AME/gross energy, regardless of sNSP/tNSP content ($P < 0.01$ for all). There was an MC × sNSP/tNSP interaction for feed intake (**FI**, $P < 0.05$), denoting that in the absence of MC, the HS-fed birds had lower FI than LS-fed birds, but this difference was eliminated when MC was present. There were MC × sNSP/tNSP interactions observed for AME intake (**AMEi**) per metabolic BW (**$BW^{0.70}$**, $P < 0.05$), AMEi/N retention (**Nr**, $P < 0.01$), NE intake (**NEi**)/Nr ($P < 0.05$), retained energy (**RE**) as fat per total RE (**REf/RE**, $P < 0.01$), and N efficiency (Nr/N intake, $P < 0.05$). These interactions showed that MC application increased $AMEi/BW^{0.70}$, AMEi/Nr, NEi/Nr, and REf/RE only in the HS-fed birds, and N efficiency only in the LS-fed broilers. This study demonstrated that MC application markedly increased feed energy utilization in all diets, and increased N efficiency in birds fed an LS diet.

Key words: net energy, AME, lipogenesis, energy intake:N retention, N efficiency

INTRODUCTION

Energy is a major cost component of broiler feeds, whereby cereal grains and coproducts are the primary energy contributors. However, these vegetable ingredients, along with protein crops other than corn and soybean meal, contain high levels of non-starch polysaccharides (**NSP**), with the predominant polymers including arabinoxylan (xylose and arabinose) in wheat and β-glucan in oat and barley (Marquardt, 1997; Knudsen, 2014). These NSP are resistant to digestive enzymes in chickens, and they can be categorized into water soluble NSP (**sNSP**) or insoluble NSP (**iNSP**) (Choct, 2002; Williams et al., 2014).

The anti-nutritional effects of iNSP act as a physical barrier to enzymes. These polymers also have a cell wall encapsulating effect or cage effect, and they are involved in gut filling and dietary energy dilution (Smits and Annison, 1996; Sarmiento-Franco et al., 2000; Noblet and Le Goff, 2001; Choct et al., 2004). However, iNSP polymers have little impact on viscosity (Sarmiento-Franco et al., 2000), and have no detrimental effect on nutrient digestibility (Choct, 2002), but do have laxative properties, reducing bacterial load in the hindgut (Smits and Annison, 1996). The iNSP may also be easily degradable in the chicken gut (Choct et al., 2004), or be biologically inert (Annison, 1991), suggesting that broilers can thrive on diets containing more iNSP than is currently being used in practice (Kalmendal et al., 2011; Kheravii et al., 2017). On

the other hand, sNSP increase digesta viscosity, which interferes with lipid, protein, and starch digestion and absorption, leading to lowered feed AME (Choct and Annison, 1991; Choct et al., 2004; Knudsen, 2014). Therefore, the anti-nutritional effects of NSP are mainly attributed to the encapsulating effect by iNSP and, to a greater extent, the viscous property instigated by the sNSP (Annison and Choct, 1991; Annison, 1993; Bedford, 1997; Bautil et al., 2019).

In recognition of the negative impact of high intestinal viscosity, the application of exogenous carbohydrases has become common practice to maximize energy utilization in poultry diets. Multiple studies have established the beneficial effect of NSP degrading carbohydrases on viscosity reduction, nutrient digestibility, and performance, especially in viscous feeds (Bedford, 1997; Meng et al., 2005; Williams et al., 2014; Abdallh et al., 2020). However, the positive responses to carbohydrase use may also be due to a disruption in cell wall integrity, thus releasing encapsulated nutrients (Meng et al., 2005). The application of these enzymes can, therefore, substantially improve feed energy by enhancing carbohydrate, CP, and fat digestibility (Almirall et al., 1995). However, there is still limited information on utilization response to the energy released by carbohydrases in broiler diets containing a range of NSP concentrations. As stated earlier, sNSP is the most detrimental NSP to nutrient utilization, whereas iNSP may be beneficial to a certain degree, and both polymers are concurrently present in feedstuff but with different proportions. The ratio of sNSP to total NSP (**tNSP**) might be an important indication of the NSP feature of a diet when the sNSP is in a range of moderate levels. Carbohydrases target both sNSP and iNSP, and the resulting degradation effects by these enzymes on bird performance might be different for sNSP and iNSP. Therefore, the ratio sNSP/tNSP would be a better indication of the diet in terms of the NSP content. Thus, the purpose of this study was to determine how multi-carbohydrases (**MC**) and the dietary sNSP/tNSP ratio affect energy partitioning, energy utilization efficiency, nitrogen (**N**) balance, and bird performance, and their interactions, in broilers using a closed-circuit calorimetric system.

MATERIALS AND METHODS

The study employed a factorial arrangement of treatments with a random blocked design (blocked by run), with 4 dietary treatments replicated 8 times. Factors were MC—no or yes; and sNSP/tNSP—low (**LS**) vs. high (**HS**). The protocol used in the study was approved by the Animal Ethics Committee of the University of New England (approval number AEC17-066).

Animal Management and Gas Exchange Measurement

A total of 64 as-hatched broiler chicks (1-day-old Ross 308 strain) were obtained from a local hatchery (Baiada Pty Ltd., Tamworth, NSW, Australia) in 2 runs (n = 32 per run), and they were feather sexed on arrival. Bird brooding and husbandry practices followed Aviagen (2014b) Ross 308 management guidelines. The net energy (**NE**) experiment was run twice using 16 closed respiratory chambers per run with 2 birds, a male and a female, per chamber. The respiratory chambers used in this study have been detailed by Wu et al. (2019). Birds were offered feed and water access ad libitum from day 0 to 28. All birds were reared on wheat-soybean meal-based feeds using a 3-phase feeding program, including a starter phase from day 0 to 10, a grower phase from day 10 to 18, and a grower-finisher phase from day 18 to 28 (Table 1). From day 0 to 21, birds were housed in floor pens in a climate-controlled room. Birds were then assigned to 16 respiratory chambers housed in a climate-controlled room and provided a 4-day acclimatization period from day 21 to 25, with chambers open and pumps running. Data for energy partitioning and feed conversion ratio were collected daily over a 3-day period from day 25 to 28. Chambers were closed during the NE run and were opened each day for data collection, including to weigh the birds and feed, weigh O_2 cylinder, and replace KOH, measurement of CO_2 and O_2 in the chambers, total excreta collection to evaluate AME, as well as collect KOH subsamples for CO_2 recovery. On day 28, all birds were euthanized using electrical stunning followed by cervical dislocation to collect digesta samples, which were pooled per chamber. The digesta samples were collected on ice and frozen at $-20°C$ immediately after collection, and then freeze-dried, ground (through a 0.5-mm screen), and stored in sealed plastic containers at room temperature for later analysis.

Experimental Diets

All diets were formulated to meet or exceed the nutrient specifications of as-hatched Ross 308 chicks (Aviagen, 2014a). The standard ileal digestible amino acids were calculated and adjusted based on a 3-phase feeding program (Adedokun et al., 2008; Zeitz et al., 2019). The formulation of all dietary treatments was based on ingredient and nutrient proportions shown in Table 1. Common diets were composed of 12.8 MJ/kg AME and 240 g/kg CP for the starter phase, and 13.4 MJ/kg AME and 209 g/kg CP for grower phase (calculated). The basal grower-finisher category contained 14.6 MJ/kg AME and 233 g/kg CP for the LS diet, and 15.5 MJ/kg AME and 222 g/kg CP for the HS diet (analyzed). The LS-based diet had an analyzed sNSP/tNSP ratio of 9.12% compared to the HS diet with 16.3% sNSP/tNSP. The levels of sNSP/tNSP ratio in the experimental diets were obtained using feed ingredients. This ratio was reduced in the LS diet using wheat bran (56.0 g/kg of complete feed), and increased in the HS diet by including 110 g/kg oat bran.

A combination of MC (Rovabio Advance T-Flex in powder form, thermostable during feed pelleting up to

Table 1. Compositions of the common starter, common grower, and basal grower-finisher diets.

Ingredient, g/kg as-fed	Common starter	Common grower	Basal dietary treatments LS diet	HS diet
Wheat	585	673	597	555
Soybean meal	343	253	256	244
Wheat bran	-	-	56.0	-
Oat bran	-	-	-	110
Canola oil	32.9	38.0	50.0	50.0
Limestone	11.3	10.6	11.2	10.2
Dicalcium phosphate, 18% P 21% Ca	15.6	14.0	12.6	14.8
Salt	3.57	1.67	3.07	1.78
Na bicarbonate	-	2.00	-	2.00
TiO$_2$	-	-	5.00	5.00
Trace mineral premix[1]	1.00	1.00	1.00	1.00
Vitamin premix[2]	0.700	0.700	0.700	0.700
Choline Cl 70%	0.514	0.536	0.681	0.826
L-lysine HCl 78.4	2.98	2.68	2.90	2.69
D,L-methionine	2.79	2.04	2.37	2.22
L-threonine	0.935	0.723	0.927	0.738
Carbohydrase enzymes[3]	0.050	0.050	-	-
Nutrient, g/kg unless otherwise indicated	Calculated nutrients		Analyzed nutrients	
AME, MJ/kg	12.8	13.4	14.6	15.5
CP	240	209	233	222
sNSP	36.1	31.4	6.53	9.71
iNSP	92.8	90.2	65.1	49.8
tNSP	129	122	71.6	59.5
sNSP:tNSP, g/kg:g/kg	0.280	0.259	0.091	0.163
			Calculated nutrient	
SID amino acids				
Lysine	12.8	10.6	10.6	10.6
Methionine	5.81	4.73	4.92	4.84
Threonine	9.20	6.80	6.80	6.80
Methionine + cysteine	8.10	7.90	7.90	7.90
Calcium	8.80	8.00	8.00	8.00
Phosphorus available	4.40	4.00	4.00	4.00

Abbreviations: HS, high sNSP/tNSP; iNSP, insoluble NSP; LS, low sNSP/tNSP; NSP, non-starch polysaccharides; SID, standard ileal digestibility; sNSP, soluble NSP; tNSP, total NSP.

[1]Formulated to supply 23 mg copper, 1.79 mg iodine, 57 mg iron, 171 mg manganese, 0.43 mg selenium, and 143 mg zinc per kg finished feed.

[2]Formulated to supply 5040 mg retinol, 17.5 mg cholecalciferol, 105 mg tocopheryl acetate, 4 mg menadione, 4 mg thiamine, 11 mg riboflavin, 77 mg niacin, 18 mg pantothenate, 7 mg pyridoxine, 0.35 mg biotin, 3.0 mg folate, 0.02 mg cyanocobalamin per kg of finished feed.

[3]Rovabio Advance (xylanase, β-glucanase, and arabinofuranosidase).

90°C) based on xylanase, β-glucanase, and arabinofuranosidase, and produced by *Talaromyces versatilis* fermentation was used. The enzymes were applied at the inclusion rate of 50 g/tonne of formulated feed to provide a minimum of 1,250 and 860 visco-units/g of xylanase and β-glucanase, respectively, and 9,250 units of arabinofuranosidase in a complete feed. A β-glucanase or xylanase visco-unit corresponds to an enzyme amount hydrolyzing barley β-glucan and wheat arabinoxylan, respectively, for lowering the viscous fluidity up to 1 arbitrary unit/min at 30°C and pH 5.5, whereas a unit of arabinofuranosidase is defined as the amount of enzyme which, at 50°C and pH 4, can reduce the viscosity of wheat arabinoxylan to liberate 1 nmol arabinose/min (Cozannet et al., 2017).

Chemical Analysis and Calculations

The calculation of AME, heat production (**HP**), NE, retained energy (**RE**), and NE used in this study followed the method described by Wu et al. (2019). Briefly, gross energies (**GE**) in the feed and excreta were measured using an adiabatic bomb calorimeter (C7000, IKA Werke GmbH and Co., Staufen, Germany). Feed AME was obtained by dividing the difference between feed GE and excreta GE with feed intake (**FI**). Energy retention was obtained by subtracting the HP calculated using the measured respiratory gas exchange (CO_2 expired and O_2 inhaled) from AME intake (**AMEi**). The proportion of energy retained as fat (**REf**) per total RE (**REf/RE**) was calculated using the following equations:

$$\text{Energy retained as protein (REp, kJ / g)} = \text{N retention (Nr)} \times 6.25 \times 23.8 \tag{A}$$

$$REf/BW^{0.70} \ (kJ \ / \ kg) = RE/BW^{0.70} \ (kJ \ / \ kg \ [B]) - REp/BW^{0.70} \ (kJ \ / \ kg) \qquad (C)$$

$$REf/RE \ (kJ \ / \ kg : kJ \ / \ kg) = (C) \ / \ (B)$$

NE was calculated by dividing the sum of RE and fasting HP with FI. To take into account the effect of energy balance per BW, energy balance variables (AMEi,

automatic N analyzer (LECO Corporation, St. Joseph, MI), and for TiO_2 concentration using the method described by Short et al. (1996). Apparent ileal digestibility coefficient (**AIDC**) was then determined using the following equation:

$$AIDC = 1 - [(\text{digesta nutrient (g/kg DM)} / \text{ digesta } TiO_2 \text{ (g/kg DM)}] /$$
$$[(\text{diet nutrient (g/kg DM)} / \text{ Diet } TiO_2 \text{ (g/kg DM)}]$$

HP, RE, and NE intake [**NEi**]) were standardized by scaling them on metabolic BW ($BW^{0.70}$), as proposed by Noblet et al. (2015). The exhaled CO_2 was trapped in 32% KOH during the NE run and was recovered using $BaCl_2$ precipitation method, as previously described (Annison and White, 1961; Swick et al., 2013; Wu et al., 2019).

Feed, excreta, and ileal digesta samples were analyzed for DM by drying them in an oven at 105°C until a constant weight was reached. Feed and digesta samples were analyzed for N using a LECO FP-2000

Statistical Procedures

Data means were analyzed for the significance of interactions and main effects using a 2-way ANOVA by the GLM procedure of Minitab (Minitab Inc., State College, PA). Prior to the test, all data were tested for normality distribution using Anderson–Darling test, and non-normal data were transformed using Johnson transformation to fit the normal distribution. Data were then analyzed using a 2 × 2 factorial arrangement of

Table 2. Effects of MC and sNSP/tNSP on feed energy and performance from day 25 to 28.

Treatments		Feed energy value, kJ/g DM			Energy utilization, %		WG, g/b/day	FI, g/b/day (DM)	FCR (DM)	Energy intake/ WG, kJ/g	
		AME	AMEn	NE	AME/GE	NE/AME				AMEi	NEi
MC	sNSP/tNSP										
No	LS	14.63	13.80	11.27	74.48	76.78	92.94	122.6[a]	1.328	19.34	14.84
No	HS	15.47	14.67	12.01	77.31	77.63	80.46	107.5[b]	1.342	20.76	16.12
Yes	LS	15.14	14.27	11.67	76.65	77.10	88.72	114.6[a,b]	1.295	19.52	15.09
Yes	HS	15.86	15.07	12.36	79.86	77.92	87.89	114.2[a,b]	1.304	20.61	16.10
Main effects											
MC	No	15.05[b]	14.24[b]	11.64[b]	75.89[b]	77.21	86.70	115.0	1.335	20.05	15.48
	Yes	15.50[a]	14.67[a]	12.01[a]	78.25[a]	77.51	88.30	114.4	1.299	20.07	15.59
sNSP/tNSP	LS	14.88[b]	14.04[b]	11.47[b]	75.56[b]	76.94	90.83	118.6	1.312	19.43[b]	14.97[b]
	HS	15.66[a]	14.87[a]	12.18[a]	78.59[a]	77.78	84.17	110.8	1.323	20.69[a]	16.11[a]
Pooled SEM		0.0980	0.0990	0.0922	0.4440	0.2790	1.850	1.810	0.0166	0.2520	0.1990
P-value											
MC × sNSP/tNSP		0.610	0.766	0.822	0.749	0.984	0.093	0.031	0.918	0.638	0.609
MC		0.001	0.001	0.004	0.001	0.597	0.634	0.843	0.163	0.958	0.683
sNSP/tNSP		<0.001	<0.001	<0.001	<0.001	0.149	0.057	0.024	0.659	0.001	<0.001

[a,b]Means within a column not bearing the same superscripts differ significantly ($P < 0.05$).

Abbreviations: AMEi, AME intake; AMEn, AME corrected for nitrogen; FCR, feed conversion ratio; FI, feed intake; GE, gross energy; HS, high sNSP/tNSP (MJ/kg:%); LS, low sNSP/tNSP (MJ/kg:%); MC, multi-carbohydrases; NE, net energy; NEi, NE intake; NSP, non-starch polysaccharides; sNSP, soluble NSP; tNSP, total NSP; WG, weight gain.

Table 3. Effects of MC and sNSP/tNSP on energy and N balance and digestibility (day 25–28).

Treatments		Energy balance, kJ/kg BW$^{0.70}$					N balance, g/b/day				
		HP	RE	AMEi	NEi	REf/RE, %	Ni	Nr	Nr/Ni, %	RQ	CP AIDC, %
MC	sNSP/tNSP										
No	LS	774.2	629.0	1403^{a,b}	1,078	45.08^{b,c}	4.562	2.918	64.18^{b}	1.020	80.85
No	HS	749.5	591.8	1341^{b}	1,042	48.92^{b}	3.822	2.501	65.98^{a,b}	1.032	84.91
Yes	LS	755.4	591.7	1347^{a,b}	1,042	42.81^{c}	4.297	2.896	67.42^{a}	1.027	83.48
Yes	HS	761.2	666.2	1427^{a}	1,116	53.63^{a}	3.981	2.607	65.56^{a,b}	1.046	84.85
Main effects											
MC	No	761.8	610.4	1,372	1,060	47.00	4.192	2.710	65.08	1.026	82.88
	Yes	758.3	628.9	1,387	1,079	48.22	4.139	2.752	66.49	1.037	84.16
sNSP/tNSP	LS	764.8	610.3	1,375	1,060	43.95	4.430^{a}	2.907^{a}	65.80	1.024^{b}	82.16^{b}
	HS	755.4	629.0	1,384	1,079	51.27	3.902^{b}	2.552^{b}	65.77	1.039^{a}	84.88^{a}
Pooled SEM		5.190	17.30	20.30	17.30	0.9780	0.0771	0.0506	0.4940	0.0058	0.5640
P-value											
MC × sNSP/tNSP		0.116	0.058	0.036	0.059	0.007	0.084	0.430	0.037	0.733	0.203
MC		0.708	0.516	0.645	0.502	0.311	0.655	0.603	0.089	0.060	0.223
sNSP/tNSP		0.325	0.513	0.776	0.506	<0.001	<0.001	<0.001	0.336	0.009	0.014

[a,b]Means within a column with different superscripts differ significantly ($P < 0.05$, Tukey pairwise comparison test).

Abbreviations: AIDC, apparent ileal digestibility coefficient; AMEi, AME intake; BW$^{0.70}$, metabolic BW; HP, heat production; HS, high sNSP/tNSP (MJ/kg:%); LS, low sNSP/tNSP (MJ/kg:%); MC, multi-carbohydrases; N, nitrogen; NEi, net energy intake; Ni, N intake; Nr, N retained; NSP, non-starch polysaccharides; RE, total energy retained; REf/RE, proportion of energy retained as fat per total energy retention; RQ, respiratory quotient; sNSP, soluble NSP; tNSP, total NSP.

treatments, with run as a covariate. Mean values were considered as a trend at a probability level of $0.05 < P < 0.10$ and significant at $P < 0.05$. Means with a significant difference were separated using Tukey's pairwise comparison test. Pearson correlation test was employed for correlation analysis.

RESULTS

Effect of MC and Diet Composition on Energy and Performance From day 25 to 28

As illustrated in Table 2, a significant different MC × sNSP/tNSP interaction ($P < 0.05$) was found for FI. This interaction demonstrated that, without MC, birds fed the LS diet had higher FI ($P < 0.05$) than those fed the HS diet, but with MC supplementation, this difference disappeared ($P > 0.05$). There was a tendency for interaction on weight gain (**WG**) ($P = 0.093$) to follow the same pattern as FI, showing a growth improvement by 13.4% in birds fed the unsupplemented LS diet compared to those fed the unsupplemented HS diet. In addition, MC supplementation increased AME, AMEn, AME/GE, and NE values ($P < 0.01$), regardless of dietary NSP composition. Additionally, these measurements were also higher ($P < 0.001$) in birds fed the HS diet compared to the LS diet, regardless of enzyme addition. However, this

Table 4. Effect of MC and sNSP/tNSP on energy to N characteristics from day 25 to 28.

Treatments		Feed energy:CP (kJ/g:%), %		Energy intake:Ni (kJ/kg:g/kg)		Energy intake:Nr (kJ/kg:g/kg)	
		AME:CP	NE:CP	AMEi:Ni	NEi:Ni	AMEi:Nr	NEi:Nr
MC	sNSP/tNSP						
No	LS	62.87	48.42	391.6	300.8	612.1^{c}	469.9^{c}
No	HS	69.61	54.04	435.3	338.0	666.3^{b}	517.3^{b}
Yes	LS	64.57	49.77	402.2	310.8	596.9^{c}	461.3^{c}
Yes	HS	72.78	56.70	453.7	354.5	692.7^{a}	541.1^{a}
Main effects							
MC	No	66.24^{b}	51.23^{b}	413.4^{b}	319.4^{b}	639.2	493.6
	Yes	68.77^{a}	53.23^{a}	427.9^{a}	332.6^{a}	644.8	501.2
sNSP/tNSP	LS	63.72^{b}	49.10^{b}	396.9^{b}	305.8^{b}	604.5	465.6
	HS	71.19^{a}	55.37^{a}	444.5^{a}	346.2^{a}	679.5	529.2
Pooled SEM		0.1740	0.6450	4.910	4.310	7.980	6.640
P-value							
MC × sNSP/tNSP		0.165	0.222	0.247	0.359	0.003	0.034
MC		<0.001	0.001	<0.001	0.001	0.369	0.120
sNSP/tNSP		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

[a–c]Means within a column with different superscripts differ significantly ($P < 0.05$, Tukey pairwise comparison test).

Abbreviations: AME:CP, AME to CP ratio; AMEi:Ni, AME intake/BW$^{0.70}$ to N intake/BW$^{0.70}$; AMEi:Nr, AMEi/BW$^{0.70}$ to N retention/BW$^{0.70}$; BW$^{0.70}$, metabolic BW; HS, high sNSP/tNSP; LS, low sNSP/tNSP; MC, multi-carbohydrases; N, nitrogen; NE:CP, net energy to CP ratio; NEi:Ni, NE intake/BW$^{0.70}$ to Ni/BW$^{0.70}$; NEi:Nr, NEi/BW$^{0.70}$ to Nr/BW$^{0.70}$; NSP, non-starch polysaccharides; sNSP, soluble NSP; sNSP/tNSP, ratio of sNSP:tNSP; tNSP, total NSP.

increase in feed energy in birds fed the HS diet resulted in higher energy (AME and NE) consumption per WG compared to that observed in birds fed the LS diet ($P < 0.01$ and $P < 0.001$, respectively). The ratio NE/AME and the feed conversion ratio were not affected ($P > 0.05$) by MC or dietary sNSP/tNSP ratio.

Impact of MC and Dietary sNSP/tNSP on Energy and N Balance

Table 3 shows that there was a different MC × sNSP/tNSP interaction ($P < 0.01$) on REf/RE, showing that MC increased REf/RE ($P < 0.01$) in birds fed the HS diet, whereas there was no MC impact on REf/RE ($P > 0.05$) in the LS-fed birds. This interaction also demonstrated that in the non-MC supplemented group there was no difference in REf/RE ($P > 0.05$) between birds fed the LS and HS diet, but with MC supplementation the HS-fed chickens presented increased REf/RE ($P < 0.01$) compared to their LS-fed counterparts. This led to a different MC × sNSP/tNSP interaction ($P < 0.05$) for AMEi/BW$^{0.70}$, demonstrating that MC increased AMEi/BW$^{0.70}$ ($P < 0.05$) only in birds reared on the HS diet. Interaction tendencies were observed for RE/BW$^{0.70}$ and NEi/BW$^{0.70}$ ($P = 0.058$ and $P = 0.059$, respectively), showing that these variables tended to follow the same pattern as seen with AMEi/BW$^{0.70}$. On the other hand, a different interaction occurred ($P < 0.05$) between MC and sNSP/tNSP for N efficiency (N retention [**Nr**]/N intake [**Ni**]), showing that MC application elevated N efficiency ($P < 0.05$) in birds fed the LS diet but not in those fed the HS diet. In addition, birds fed the LS diet displayed lower Ni and Nr ($P < 0.001$), irrespective of MC supplementation. This

diet also resulted in elevated respiratory quotient (**RQ**) and CP AIDC ($P < 0.01$ and $P < 0.05$, respectively), regardless of supplemental MC. There was no impact of MC ($P > 0.05$) or dietary composition on HP.

Effect of MC and Dietary Composition on Energy per N Characteristics

Dietary treatments and FI were analyzed for energy to CP, Ni, and Nr ratios (Table 4). MC × sNSP/tNSP interactions on energy (AME and NE) intake to Nr ($P < 0.01$ and $P < 0.05$, respectively) occurred. These interactions illustrated that MC had no impact ($P > 0.05$) on the LS diet, but did increase AMEi/Nr and NEi/Nr ($P < 0.05$) in the HS-fed birds. In addition, MC supplementation increased AME:CP, NE:CP, AMEi:Ni, and NEi:Ni ($P < 0.01$ or lower), irrespective of dietary composition. Similarly, these variables were increased in the HS diet ($P < 0.001$), regardless of MC addition.

Correlation Matrix Between Feed Energy and N

All correlation data are tabulated in Table 5. There were positive correlations ($P < 0.001$) between AME vs. CP AIDC and REf/RE (r = 0.576 and r = 0.585, respectively). This variable also had a weak positive correlation ($P < 0.05$) with Nr/Ni, RQ, and NEi/WG, with r = 0.434, r = 0.356, and r = 0.373, respectively, and a negative correlation with Ni (r = −0.581, $P < 0.001$). Feed NE followed the same correlation pattern as AME. Nr/Ni was positively correlated with RQ and CP AIDC (r = 0.404, $P < 0.05$ and r = 0.418,

Table 5. Correlations between feed energy and N characteristics.

Parameter[1]	AME	NE	Ni	Nr	Nr/Ni	REf/RE	RQ	AMEi/WG	NEi/WG	CP dc	AMEi:Nr
NE	0.895***										
Ni	−0.581***	−0.513**									
Nr	−0.371*	−0.294	0.904***								
Nr/Ni	0.434*	0.470*	−0.293	0.143							
REf/RE	0.585***	0.718***	−0.102	−0.093	−0.027						
RQ	0.356*	0.440*	−0.131	0.063	0.404*	0.529**					
AMEi/WG	0.305	0.210	−0.415*	−0.536**	−0.220	0.047	−0.517**				
NEi/WG	0.373*	0.391*	−0.442*	−0.524**	−0.122	0.209	−0.423	0.958***			
CP dc	0.576***	0.458**	−0.564***	−0.416*	0.418*	0.185	0.202	0.129	0.141		
AMEi:Nr	0.595**	0.517**	−0.481**	−0.650***	−0.370†	0.637***	0.044	0.576**	0.591**	0.325†	
NEi:Nr	0.628***	0.663***	−0.479**	−0.603**	−0.260	0.756***	0.123	0.515**	0.609***	0.317†	0.961***

Abbreviations: AME (MJ/kg DM), AME; AMEi:Nr (kJ/kg:g/kg), AMEi/BW$^{0.70}$ to N retention/BW$^{0.70}$ ratio; AMEi/WG (kJ/g), AME intake/WG; CP dc (%), CP digestibility coefficient; N, nitrogen; NE (MJ/kg DM), net energy; NEi:Nr/BW$^{0.70}$ (kJ/kg:g/kg), NEi/BW$^{0.70}$ to Ni/BW$^{0.70}$ ratio; NEi/WG (kJ/g), NE intake/WG; Ni (g/d), N intake; Nr (g/d), N retained; Nr/Ni (%), N efficiency; REf/RE (%), energy retained as fat per total energy retained; RQ, respiratory quotient.

[1]Probability values are indicated as *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, and †$P < 0.10$ (tendency).

$P < 0.05$, respectively). There were also positive correlations between REf/RE vs. RQ, AMEi:Nr, and NEi:Nr with r = 0.529, $P < 0.01$, r = 0.637, $P < 0.001$, and r = 0.756, $P < 0.001$, respectively. In addition, AMEi/WG was negatively correlated with Nr (r = −0.536, $P < 0.01$), and NEi/WG followed the same pattern as AMEi/WG.

DISCUSSION

The use of carbohydrases to improve feed energy and bird performance by removing the negative impact of NSP is common practice (Bedford, 1996; Abdallh et al., 2020). However, the enzymatic activities and bird responses to energy released by the enzymes may differ depending on dietary content in sNSP and iNSP. Therefore, the present study aimed to assess the extent to which an MC supplement can improve energy and bird responses to energy released by the enzymes in isoenergetic diets containing low or high sNSP/tNSP ratios. As expected, the data from this study demonstrated that the application of MC increased feed AME and NE values, irrespective of feed composition. An increase in feed AME as a result of supplemental carbohydrases in wheat-based diets is in agreement with previous findings (Cozannet et al., 2017, 2019; Abdallh et al., 2020).

Based on the results of this study, bird responses to the energy released by MC on energy utilization and N efficiency differed depending on the dietary sNSP/tNSP content. Firstly, energy utilization for lean muscle or fat deposition was dependent on the bird capacity to control energy intake. When MC was not supplemented, the HS diet reduced FI relative to the LS-fed birds, but with enzyme supplementation, this difference was eliminated by lowering FI in the LS birds and by elevating FI in the HS group. In this regard, birds fed the LS diet with MC supplementation consumed 6.5% less feed in response to energy supply, as it was recognized that broilers can control their FI to adjust their energy intake (Leeson et al., 1996). As a result, these birds had similar energy intake:Nr compared with the non-enzyme group. On the other hand, the supplemented HS-fed birds failed to adjust FI to their energy requirements in response to energy supply, as they consumed 5.3% more feed than the non-MC counterparts, which led to higher energy:Nr ratios. This small increase in FI could have been a result of viscosity reduction, as Almirall et al. (1995) have previously observed increased FI as a consequence of digesta viscosity reduction.

The energy in excess per unit of Nr induced by MC supplementation was used by the HS-fed birds for fat retention. This is evidenced by positive correlations between RQ vs. REf/RE and energy, implying that the increased feed energy was associated with increased de novo lipogenesis, a process in which bloodstream carbohydrates are converted into lipid molecules (Song et al., 2018). In partial agreement with this finding, Abdallh et al. (2020) showed that carbohydrases increased AME for lipid retention. Additionally, positive

correlations noted between REf/RE and energy intake:Nr also confirm that the increased energy consumption per Nr was a contributory factor for increased fat synthesis. The negative correlation observed between energy intake/WG and Nr suggests that an increase in Nr reduced maintenance requirements. In accordance with this, Bregendahl et al. (2002) demonstrated that protein retention reduced with elevated energy utilized by birds for growth. Moreover, the increase in AMEi/$BW^{0.70}$ occurred only when MC was supplemented in the HS diet. This increased energy consumption per $BW^{0.70}$ was due to the heightened de novo lipogenesis, as more than half of the total RE (53.6%) was used by the HS-fed birds for fat synthesis.

Secondly, bird response to N efficiency was also influenced by dietary composition. The use of MC elevated N efficiency in the LS-fed birds, whereas the enzymes showed no effect on this variable in the HS group. A positive correlation observed between N efficiency and CP digestibility implies that increased N efficiency in the supplemented LS-fed birds did not result from the N sparing effect due to suboptimal Ni (MacLeod, 1997), as Ni was similar in all diets, but probably because of the observed numerical increase in CP digestibility. In agreement with this, Nourmohammadi et al. (2018) demonstrated that birds fed a wheat-based diet supplemented with xylanase showed increased N efficiency by increasing Nr. Other studies also recorded the beneficial effect of supplementing carbohydrases in iNSP diets, which would represent the LS diet in this study. For instance, Jørgensen et al. (1996) demonstrated that, with the exception of dietary fibers from oat bran, broilers fed diets with high dietary fibers retained more energy as protein, and consequently less energy was retained as fat. It was also found that iNSP are easily degradable by carbohydrases in broilers, coupled with improved N efficiency (Choct et al., 2004). Therefore, as opposed to the HS diet, the use of MC in the LS diets may not only promote leaner birds, as indicated by higher N efficiency, but it can also be environmentally sound by alleviating damage caused by N-rich manure.

In this study, feed energy (AME and NE) and CP digestibility were higher in the HS than LS-diet category, regardless of MC supplementation. This could have been due to the oat bran that was used in this study to increase the dietary sNSP/tNSP content. As opposed to wheat bran used to lower the dietary sNSP/tNSP ratio, it is likely that oat bran contained higher quality protein and fat, coupled with sNSP that can be digested by chickens to produce energy (Hahn et al., 1990; Pettersson and Åman, 1992). This is in accordance with the findings of Jørgensen et al. (1996) who stated that oat utilization by poultry is high. This could explain the lack of MC impact on nutrient digestibility of the HS diet, which is in agreement with results reported by Bedford (1997) who explained that carbohydrase effect is exhibited in diets that are not rich in nutrient density. However, the energy released by the HS diet in this study was not cost effective in terms of energy intake per unit of bird growth. This was likely due to an increase in RQ,

meaning that more energy derived from this diet was synthesized into fat, which requires high energy intake per unit of growth (Close, 1990). Moreover, the HS diet reduced Ni and Nr, regardless of enzyme supplementation, presumably due to a high viscosity, which was found to have a deleterious effect on FI and protein utilization (Choct and Annison, 1991; Bedford, 1996).

In conclusion, MC supplement increases energy in diets with both low and high sNSP/tNSP ratios, and the resulting change in energy intake:Nr acts as a contributory factor in energy utilization for fat synthesis. The application of MC in low-energy density diets can improve WG in broilers fed diets containing high iNSP, such as a wheat-based diet supplemented with wheat bran, and lean muscle in birds fed diets with high sNSP content. However, a feeding trial is required for conclusive performance results.

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DISCLOSURES

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this study.

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