Acute-phase protein α -1-acid glycoprotein is negatively associated with feed intake in postpartum dairy cows

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ABSTRACT

α -1-Acid glycoprotein (AGP) is an acute-phase protein that may suppress dry matter intake (DMI), potentially by acting on the leptin receptor in the hypothalamus. Our objectives were to characterize plasma AGP concentration and associations with DMI during the transition period, and to determine the utility of AGP to identify or predict cows with low DMI. Plasma samples ($n = 2,086$) from 434 Holstein cows in 6 studies were analyzed on d -21 , -13 ± 2 , -3 , 1 , 3 , 7 ± 1 , 14 ± 1 , and 21 ± 1 relative to parturition. A commercially available ELISA kit specific for bovine AGP was validated, and 2 internal controls were analyzed on each plate with interplate variation of 15.0 and 17.3%, respectively. Bivariate analysis was used to assess the relationship between AGP and DMI. For significant associations, treatment(study) was added to the model, and quadratic associations were included in the model if significant. Plasma AGP concentration ($\pm$ SEM) increased from 213 ± 37.3 μ g/mL on d -3 to 445 ± 60.0 μ g/mL on d 14. On d 3, AGP was associated negatively with DMI in a quadratic manner for wk 1 and wk 2 and linearly for wk 3. Day 7 AGP was associated negatively with DMI in a quadratic manner for wk 2 and linearly for wk 3. Similarly, d 14 AGP was negatively associated with DMI for wk 3 and wk 4. As d 3 AGP concentration increased over the interquartile range, a calculated 1.4 (8.5%), 0.5 (2.7%), and 0.4 (1.9%) kg/d reduction in predicted DMI was detected during wk 1, 2, and 3, respectively. Using bivariate analysis, d 3 AGP explained 10% of the variation in DMI during wk 1. We

explored the clinical utility of d 3 AGP to diagnose low DMI, defined as wk 1 DMI >1 standard deviation below the mean. Receiver operating characteristic analysis identified a threshold of 480.9 μ g/mL, providing 76% specificity and 48% sensitivity (area under the curve = 0.60). Limited associations occurred between AGP and blood biomarkers; however, AGP was associated with plasma haptoglobin concentration postpartum and incidence of displaced abomasum, retained placenta, and metritis. These results demonstrate a negative association between plasma AGP concentration and DMI in early-postpartum dairy cows, although its diagnostic performance was marginal. Further investigation into whether AGP directly suppresses DMI in dairy cattle is warranted.

Key words: biomarker, appetite, periparturient cow

INTRODUCTION

Dairy cows transitioning to lactation experience systemic inflammation, with chronic subacute inflammation appearing to lead to long-term production losses (Bradford et al., 2015). Inflammation is the biological mechanism whereby the body copes with pathogenic or nonpathogenic stimuli to resolve an issue and return to homeostasis. Cytokine and acute-phase protein production are hallmarks of inflammation, with cytokine production by immune cells markedly increasing after tissue insult, further leading to production of acute-phase proteins. These compounds, as indicators of inflammatory status, have consistently been linked to poor performance in dairy cows (Humblet et al., 2006; McCarthy et al., 2016), and extensive evaluation of acute-phase proteins as biomarkers of various diseases in ruminants has been performed (Ceciliani et al., 2012). Additionally, cytokines have been directly implicated in reducing feed intake through action on the hypothalamus (Wong and Pinkney, 2004), linking

Received June 4, 2020.

Accepted August 8, 2020.

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Table 1. Experiments and number of plasma samples analyzed ($n = 2,086$) on day relative to parturition

Item	Day relative to parturition								Treatment
	-21	-13 $\pm$ 2	-3	1	3 $\pm$ 1	7 $\pm$ 1	14 $\pm$ 1	21 $\pm$ 1	
Bollatti et al., 2020	0	90	0	0	90	94	96	94	Transition-period rumen-protected choline
Carpenter et al., 2018	0	0	0	0	52	53	56	56	Postpartum sodium salicylate drench
Leno et al., 2017a	0	41	0	0	41	41	41	41	Ca/Mg source, postpartum dietary Mg concentration
Leno et al., 2017b	0	89	0	0	89	58	87	88	Prepartum DCAD level with low-K diet
Olagaray et al., 2019	0	62	0	0	0	0	59	0	Transition-period yeast fermentation product
Zenobi et al., 2018	91	85	44	87	90	90	92	92	Rumen-protected choline, prepartum dietary energy density
Total	91	367	44	87	362	336	431	371	

the immune response as an additional pathway for feed intake regulation beyond the traditional physical, endocrine, and metabolic homeostatic factors eloquently outlined by Allen and Piantoni (2013).

Orosomucoid, or α -1-acid glycoprotein (**AGP**), is a positive acute-phase protein produced primarily in the liver (Hochebied et al., 2003). The role of AGP is not entirely clear, but it is a common lipocalin that also has immunomodulatory effects, working mostly in an anti-inflammatory manner (Fournier et al., 2000; Cecilian and Lecchi, 2019). Two reports have demonstrated compelling evidence that AGP works directly to reduce feed intake in rats after intracerebroventricular infusion (Bellinger and Mendel, 1990), intraperitoneal injection, or peripheral administration (Sun et al., 2016). More mechanistically, AGP was found to bind the hypothalamic leptin receptor in mice, which in turn activates the downstream JAK2-STAT3 pathway to relay satiety signals (Sun et al., 2016). The hypothalamus is an important structure in the regulation of energy intake (Schwartz et al., 2000). However, very recent reports demonstrate a lack of effect of AGP on STAT3 signaling and feed intake in ruminants (Gregg et al., 2019; McGuckin et al., 2020).

Considering that plasma AGP concentration increases in postpartum dairy cows in a pattern similar to other acute-phase proteins (Cairoli et al., 2006; Jafari et al., 2006), and peripartum cows often exhibit depressed feed intake, we hypothesized that plasma AGP concentration is negatively related to DMI during the transition period. The objectives of this study were (1) to assess peripartum AGP concentration using a large sample size, (2) to determine the relationship between AGP and DMI in dairy cows during the transition period, (3) to assess the prognostic value of AGP relative to other biomarkers to predict cows at risk of low DMI, and (4) to assess the prognostic value of AGP to predict postpartum disease.

MATERIALS AND METHODS

Animals and Samples

Blood plasma samples ($n = 2,086$) from 434 Holstein cows were obtained from 6 previously conducted studies since 2014 at 3 institutions (Cornell University, University of Florida, and Kansas State University; Leno et al., 2017a,b; Carpenter et al., 2018; Zenobi et al., 2018; Olagaray et al., 2019; Bollatti et al., 2020). All samples were obtained ± 21 d relative to parturition, with specific sample days for each study outlined in Table 1. Individual daily feed intake data were available from all cows included in analysis. Detailed descriptions of study methodology, including analysis of other blood biomarkers, were previously published for each of these studies. Samples were analyzed in duplicate.

Sample Analysis

Initial attempts at quantifying AGP involved validating a previously reported ELISA procedure (Blees et al., 2017) modified to use a commercially available purified bovine AGP (product # G3643, lot no. 111M7401V; Sigma-Aldrich, St. Louis, MO) dissolved in distilled, deionized water for the standard curve to avoid nonspecific binding. Lack of detectability of the purified bovine AGP prompted evaluation of commercial ELISA kits (MyBioSource Inc., San Diego, CA; ICL Inc., Portland, OR) to determine whether the commercially purified AGP could be detected, and to potentially validate the kits for large-scale AGP quantification. The purified bovine AGP was not detectable on either kit, necessitating the use of commercial kit standards.

Samples for the complete analysis were analyzed using a bovine-specific ELISA kit (catalog #E-10AGP; ICL Inc.) according to manufacturer specifications at a dilution of 1:20,000. The kit uses a lyophilized puri-

Table 2. Spike and recovery results of the bovine ELISA kit (ICL Inc., Portland, OR)

Spike (ng/mL)	AGP concentration ¹ (ng/mL)		Recovery (%)	SD ²
	Expected	Observed		
5	26.3	29.0	110.3	2.1
10	31.3	32.7	104.6	2.7
20	41.3	38.3	92.9	0.7

¹AGP = α -1-acid glycoprotein.²Standard deviation of the observed concentrations, performed in triplicate.

fied bovine AGP from serum as a standard curve. The 4-step dilution was performed via a robotic multichannel pipetting system (Integra Viaflo Assist, Integra Biosciences Corp., Hudson, NH), using the diluent buffer provided in the kit. Assay performance was assessed using spike and recovery and linearity of dilution techniques. Recovery of AGP was determined by spiking a known quantity of the standard into diluted sample (Table 2). A linearity of dilution experiment was conducted to test the behavior of the samples at various concentrations in the range of the standard curve by serially diluting plasma in the assay buffer (Table 3). According to the results compared with the linear portion of the standard curve, plasma samples were diluted 1:20,000 to achieve approximately 20 ng/mL final concentration. Two internal control samples were analyzed in duplicate on each plate, with intra-assay coefficient of variation (CV) of 3.8 and 3.0% and interassay CV of 15.0 and 17.3%, respectively, for each sample.

Statistical Analysis

Plasma AGP mean concentration on each sampling date was obtained by using repeated measures in the Mixed procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC), with the model including the random effect of study. Data were $\log_{10}$ transformed to achieve normality and reverse transformed to obtain means. Bivariate linear regression, and subsequently linear and quadratic regression, was used to assess the relationship between AGP and DMI in subsequent weeks using JMP

(version 13.0, SAS Institute Inc.). DMI was averaged over a 3-d period following each blood sampling time point prepartum (d -21, -12 $\pm$ 3, and -3), except for blood sample time point d -21 for which DMI was averaged over d -15 to -13. For postpartum data, DMI was averaged over a 1-wk period for each week postpartum. Days used to calculate DMI averages for each week relative to parturition are outlined in Table 4. For significant bivariate associations, the following linear and quadratic model was used to further analyze the association between plasma AGP concentration and DMI:

$$Y_{ij} = \mu + A + A \times A + T_i + e_{ij},$$

where μ is the overall treatment mean, A is the linear effect of plasma AGP concentration, $A \times A$ is the quadratic effect of plasma AGP concentration, T_i is the random effect of unique treatment within each study ($i = 1$ to 19), and e_{ij} is the residual error ($j = 1$ to 30). Analysis of the association between plasma AGP concentration and concentration of other blood biomarkers was conducted with the same model. Quadratic effects were removed from the model when $P > 0.10$.

Residuals were analyzed for normality, and data were natural log-transformed when appropriate. Studentized residuals were evaluated, and outliers >3.5 or <-3.5 were excluded from analysis. Significance was declared at $P < 0.05$, and tendencies when $P < 0.10$.

Nominal logistic modeling was used to determine the association between plasma AGP concentration

Table 3. Linearity of dilution results for AGP¹ using bovine ELISA kit (ICL Inc., Portland, OR)

Dilution rate	AGP concentration (ng/mL)			SD ²
	Observed	Expected	% of expected	
1:5,000	88.5	—	—	11.5
1:10,000	36.4	44.2	82.2	0.1
1:20,000	20.9	18.2	115.2	1.2
1:40,000	10.7	10.5	102.5	0.7
1:80,000	5.1	5.4	95.4	0.4

¹AGP = α -1-acid glycoprotein.²Standard deviation of the observed concentrations, performed in duplicate.

Table 4. Association between plasma AGP concentration¹ (natural log $\mu\text{g/mL}$) and DMI (kg/d); significant linear associations were subsequently analyzed with quadratic and linear regression, including random effect of treatment(study) in the model

Plasma AGP sample day	Week of DMI	<i>P</i> -value ²		Intercept	Parameter estimate	<i>R</i> ²	N	RMSE ³
		Linear	Quadratic					
−21	−2 ⁴	0.09	0.07	−31.37	16.14 −1.50	0.15	82	2.08
−13 ± 2	−2	0.10		13.96	−0.53	0.33	348	3.43
	1 ⁵	0.22		18.87	−0.46	0.16	367	3.27
	3	0.46		21.90	−0.25	0.21	253	3.14
	4	0.80		22.02	−0.09	0.28	291	2.88
3 ± 1	1	0.02	0.01	−2.50	8.36 −0.87	0.27	355	3.21
	2 ⁶	0.07	0.03	3.25	6.61 −0.67	0.24	318	3.08
	3 ⁷	<0.001		26.81	−1.08	0.27	350	2.92
7 ± 1	2	0.02	<0.01	6.54	5.86 −0.63	0.32	330	2.91
	3	<0.001		21.53	−0.002	0.30	327	2.80
14 ± 1	3	<0.001		28.49	−1.31	0.25	423	3.06
	4 ⁸	<0.001		28.54	−1.15	0.31	358	2.76
21 ± 1	4	0.001		27.30	−0.97	0.30	363	2.77

¹AGP = α -1-acid glycoprotein.²All data were transformed using natural log to achieve normality and data are presented in the transformed state.³RMSE = root mean squared error.⁴DMI averaged over 3 d following plasma sample date (d −13 ± 2).⁵DMI averaged over d 1 to 7.⁶DMI averaged over d 8 to 14.⁷DMI averaged over d 15 to 21.⁸DMI averaged over d 22 to 28.

on a given day and incidence of postpartum disease using the fixed effect of AGP and the random effect of study. From this information, we used receiver operating characteristic (**ROC**) analysis using JMP to assess the ability of AGP to predict disease incidence for diseases presenting sufficient sample size (sensitivity and specificity = 0.70; marginal error = 0.20; α = 0.05; Hajian-Tilaki, 2014; Bujang and Adnan, 2016), based upon previously reported rates of disease and culling (Smith et al., 2000; McLaren et al., 2006; Berge and Vertenten, 2014).

We also carried out ROC analysis to determine the utility of d 3 AGP for detecting cows with low feed intake. Day 3 AGP concentration was used because the association between AGP and DMI was most significant early in the postpartum period. Low feed intake was defined as 1 standard deviation (**SD**) less than the mean wk 1 DMI. For all ROC analyses we determined the threshold AGP concentration offering the optimal balance between sensitivity and specificity, determined based on the concentration offering the greatest sum of calculated sensitivity + specificity.

A proportional hazards regression model was constructed using the fixed effects of AGP and study to determine the association of AGP concentration with risk of culling by 280 DIM. Furthermore, the concentration of AGP was classified as being above or below the threshold determined from ROC analysis to construct survival curves assessing the association of peripartum AGP with culling by 280 DIM. The cutoff of 280 d was chosen because, for 2 of the studies (Zenobi et al., 2018; Bollatti et al., 2020), representing half of the data, information was available only up to 280 DIM, limiting the ability to evaluate the full lactation on all animals.

RESULTS

Prepartum AGP concentrations were relatively stable for d −21 ($248 \pm 35.0 \mu\text{g/mL}$), d −13 ($223 \pm 30.2 \mu\text{g/mL}$), d −3 ($212 \pm 30.2 \mu\text{g/mL}$), and d 1 ($273 \pm 37.4 \mu\text{g/mL}$; Figure 1). Plasma AGP concentration increased beginning on d 3 ($377 \pm 51.0 \mu\text{g/mL}$) before numerically increasing to a peak on d 14 ($445 \pm 59.0 \mu\text{g/mL}$) and declining numerically to d 21 (387 ± 52.2

$\mu\text{g/mL}$). Plasma concentrations of AGP on d 3 to d 21 were significantly greater than AGP on any day from d -21 to d 1 ($P < 0.001$). Results for associations between AGP and DMI are presented in Table 4. All associations between the variables were negative over the range of observed values (accounting for the quadratic nature of some relationships). Concentration of AGP on d -21 was negatively related in a quadratic manner with DMI of wk -2 ($P = 0.07$). On d 3, AGP was negatively associated with DMI on wk 1 and wk 2 in a quadratic fashion ($P = 0.01$; $P = 0.03$) and was negatively associated in a linear manner with wk 3 DMI ($P < 0.001$). The AGP concentration on d 7 had a negative quadratic association with wk 2 DMI ($P < 0.01$) and was negatively associated with wk 3 DMI ($P < 0.001$). Day 14 AGP concentration was negatively associated with DMI in a linear manner for both wk 3 and wk 4 ($P < 0.001$). The AGP concentration on d 21 was negatively associated with DMI in wk 4 ($P = 0.001$).

Using the intercepts and parameter estimates shown in Table 4, predicted DMI changes over the interquartile range of d 3 AGP were calculated. As d 3 AGP concentration increased over the interquartile range, a calculated 1.4 (8.5%), 0.5 (2.7%), and 0.4 (1.9%) kg/d reduction in predicted DMI was detectable during wk

Figure 1. Box and whisker plots and outliers of plasma α -1-acid glycoprotein (AGP) concentration of peripartum dairy cows. Refer to Table 1 for number of samples analyzed at each time point. Data were transformed using $\log_{10}$ to achieve normality, and reverse-transformed data are shown. Symbols above each day indicate difference from postpartum time points (* = different from d-1 to d-21 time points, $P < 0.001$; † = different from d-3 to d-21 time points, $P < 0.001$). The box indicates the 25th percentile, median, and 75th percentile, and whiskers represent data within 1.5 times the interquartile range. Black dots are outliers.

Figure 2. Plasma α -1-acid glycoprotein (AGP) concentration on d 3 postpartum is negatively associated with early-lactation DMI. The reduction in DMI calculated over the interquartile range of d 3 plasma AGP concentration (222 to 488 $\mu\text{g/mL}$; panel A) and the bivariate fit of d 3 AGP ($\mu\text{g/mL}$) and wk 1 DMI (panel B).

1, 2, and 3, respectively (Figure 2A). Using bivariate analysis, d 3 AGP explained 10.0% of the variation in DMI during wk 1 (Figure 2B). Receiver operating characteristic analysis identified a threshold of 480.9 $\mu\text{g/mL}$, providing 76% specificity and 48% sensitivity [Figure 3; area under the curve (AUC) = 0.60] in detecting low feed intake.

Results for associations between AGP and other blood plasma biomarkers can be found in Supplemental Tables S1 to S8 (<https://doi.org/10.3168/jds.2020-19025>). Plasma AGP concentration on d 1 tended to be positively associated with plasma glucose concentration on d 7 ($P = 0.06$) and d 14 ($P = 0.07$). On d -3, plasma AGP was positively associated with d 21 free fatty acids ($P = 0.04$) and tended to be positively associated with d 14 free fatty acids ($P = 0.08$), whereas d 7 AGP tended to be negatively associated with d 14 free fatty acids ($P = 0.07$). Plasma AGP on d -13 was negatively associated with insulin concentration at the same time point ($P = 0.04$). The strongest associations

Figure 3. Receiver operating characteristics curve (jagged line) that shows the critical threshold (intersection with solid line) for plasma α -1-acid glycoprotein (AGP) concentration on d 3 (480.9 $\mu\text{g/mL}$), predicting low DMI for wk 1 (area under the curve = 0.60). The critical threshold AGP concentration offered the optimal balance between sensitivity and specificity, determined based on greatest sum of calculated sensitivity and specificity. Low DMI was defined as wk 1 DMI less than 1 SD below the mean (<12.5 kg/d; $n = 54$).

were between AGP and BHB and haptoglobin. On d -13 , AGP was negatively associated with BHB ($P = 0.02$), whereas d 7 AGP concentration was positively associated with BHB on d 7 ($P < 0.01$; Table 5). The concentrations of AGP and haptoglobin were positively associated on d 3, 7, and 14 ($P \leq 0.03$; Table 5), and d 7 AGP was also associated with d 14 haptoglobin ($P < 0.01$; Table 5).

Strong associations were detectable between postpartum AGP concentration and transition cow diseases.

The concentration of AGP on d 3 to 14 ($P < 0.001$) and d 21 ($P = 0.02$; Table 6) was positively associated with postpartum incidence of retained placenta. In a similar fashion, AGP on d 3 to 21 ($P < 0.001$; Table 6) was positively associated with incidence of metritis. On d 7 ($P = 0.03$), 14 ($P = 0.04$), and 21 ($P < 0.001$; Table 6), AGP was positively associated with hyperketonemia. On d 21, AGP was positively associated with mastitis ($P = 0.03$). Furthermore, AGP was positively associated on d 1 ($P = 0.04$) with culling by 280 DIM. Overall, 19% of at-risk cows left the herd by 280 DIM, and proportional hazard analysis revealed that increased AGP concentration over the interquartile range (432 vs. 271 $\mu\text{g/mL}$) on d 1 was associated ($P = 0.02$) with a 15% decreased risk of culling through 280 DIM (0.85 hazard ratio, 95% CI: 0.82 to 0.95). Survival curves were constructed for cohorts using the optimal threshold for d 1 AGP concentration (351 $\mu\text{g/mL}$) determined via ROC analysis, but no evidence of difference was detectable between cows above and below the threshold ($P = 0.11$ Wilcoxon test; data not shown).

DISCUSSION

To accomplish most of our objectives for this study, we first had to identify an accurate, high-throughput method for determination of AGP concentration in bovine plasma. To date, most of the quantification of circulating AGP has been conducted via radial immunodiffusion assays (Jafari et al., 2006; Eckersall et al., 2007), immunoturbidimetric assay (Bence et al., 2005; Mooney et al., 2006), or gel electrophoresis methods (Ling et al., 1996; Cairoli et al., 2006), or by precipitating the protein and measuring absorbance (Lewis et al., 1989), none of which are suitable for analyzing large quantities of samples. More recent efforts have resulted in development of ELISA procedures to quantify AGP (Agra et al., 2017; Caperna et al., 2017), with only 1

Table 5. Association between plasma α -1-acid glycoprotein (AGP) concentration (natural log $\mu\text{g/mL}$) and plasma BHB (μM) and haptoglobin (Hp) concentrations¹ for select sampling points postpartum

AGP plasma sample day	Variable sample day	<i>P</i> -value ²		Intercept	Parameter estimate	<i>R</i> ²	<i>N</i>	RMSE
		Linear	Quadratic					
7 $\pm$ 1	BHB d 7	<0.01		54.1	113.3	0.09	322	425.8
3 $\pm$ 1	Hp d 3	<0.001		$-1,439.5$	272.1	0.24	50	233.1
7 $\pm$ 1	Hp d 7	<0.001		$-1,875.8$	406.6	0.32	143	518.0
7 $\pm$ 1	Hp d 14	<0.01		-532.8	149.6	0.28	140	318.3
14 $\pm$ 1	Hp d 14	0.03		3,598.4	$-1,286.8$	0.31	145	337.0
			0.02		123.2			

¹Data for haptoglobin from Carpenter et al. (2018) were reported as $\mu\text{g/mL}$, and data from Bollatti et al. (2020) and Zenobi et al. (2018) were reported as optical density. Differences between concentration reporting methods are accounted for with the random effect of treatment (study) in our model.

²All data for AGP concentration were transformed using natural log to achieve normality, and data are presented in the transformed state.

study quantifying bovine plasma AGP (McGuckin et al., 2020). For this reason, we tested several ELISA kits and found the one used in this study to be acceptable for linearity and recovery, and it most closely aligned with previously reported bovine plasma AGP concentrations using other techniques (Horadagoda et al., 1999; Jafari et al., 2006; Ceciliani et al., 2012).

Changes in Peripartum AGP Concentration

Our first objective was to assess AGP concentrations on a large sample size of peripartum dairy cows. The pattern of plasma AGP concentration shifts during

the transition period in this study is similar to those reported for other positive acute-phase proteins, such as haptoglobin, LPS binding protein, and serum amyloid A (Jafari et al., 2006; Abuajamieh et al., 2016; McCarthy et al., 2016). The concentration of AGP significantly increases postpartum by d 3, reaching an apparent peak at 14 DIM before numerically trending toward prepartum concentrations. This is similar to the pattern shown by McGuckin et al. (2020), where postpartum concentrations of AGP were significantly greater than prepartum concentrations, but lacked evidence of differences across time points in the first 3 wk postpartum. In another study, plasma AGP peaked

Table 6. Association between α -1-acid glycoprotein (AGP; natural log $\mu\text{g/mL}$) and incidence of postpartum disease and culling in transition dairy cows; all associations were positive unless otherwise denoted by a superscript

Disease	AGP sample day (relative to parturition)							
	−21	−13 ± 2	−3	1	3 ± 1	7 ± 1	14 ± 1	21 ± 1
Cows at risk, no.	91	360	44	87	306	280	369	310
Hyperketonemia								
<i>P</i> -value								
AGP ¹	0.10	0.20	0.13	0.61	0.15	0.03	0.04	<0.001
Study	—	0.69	—	—	0.12	0.08	0.15	0.04
Cases, ² no.	20	91	10	18	78	69	91	77
AUC ³	0.60	0.59	0.51	0.50	0.59	0.62	0.60	0.66
AGP threshold, ⁴ $\mu\text{g/mL}$	246	258	211	246	531	165	464	275
Retained placenta								
<i>P</i> -value								
AGP	0.81 ⁵	0.16 ⁵	0.87 ⁵	0.72 ⁵	<0.001	<0.001	<0.001	0.02
Study	—	<0.01	—	—	0.46	0.69	<0.001	0.25
Cases, no.	19 ⁶	33	5 ⁶	17 ⁶	37	30	38	40
AUC	—	0.70	—	—	0.74	0.77	0.83	0.69
AGP threshold, ⁴ $\mu\text{g/mL}$	—	77	—	—	496	853	477	431
Metritis								
<i>P</i> -value								
AGP	0.63 ⁵	0.71 ⁵	0.96	0.81 ⁵	<0.001	<0.001	<0.001	<0.001
Study	—	0.19	—	—	0.08	0.01	0.07	0.13
Cases, no.	19 ⁶	37	5 ⁶	18 ⁶	40	34	41	41
AUC	—	0.61	—	—	0.78	0.86	0.81	0.74
AGP threshold, ⁴ $\mu\text{g/mL}$	—	330	—	—	401	643	365	548
Mastitis								
<i>P</i> -value								
AGP	0.95 ⁵	0.55	0.11	0.31	0.25	0.11	0.10	0.03
Study	—	<0.001	—	—	<0.01	<0.01	<0.001	0.03
Cases, no.	23	51	10	24	49	45	49	49
AUC	0.54	0.74	0.63	0.53	0.67	0.74	0.74	0.7
AGP threshold, ⁴ $\mu\text{g/mL}$	223	152	291	236	164	383	1,216	413
Culling by 280 DIM								
<i>P</i> -value								
AGP	0.10 ⁵	0.15 ⁵	0.84	0.04 ⁵	0.99 ⁵	0.71	0.52	0.98
Study	—	0.10	—	—	0.07	0.13	0.11	0.07
Culled, no.	15	61	8	14	60	51	68	58
AUC	0.61	0.62	0.58	0.63	0.61	0.62	0.62	0.61
AGP threshold, ⁴ $\mu\text{g/mL}$	183	50	291	350	419	410	182	357

¹Data for AGP concentration were transformed using natural log to achieve normality, and data are presented in the transformed state.

²Number of observations of animals in the data set for a particular sampling day that were diseased at some point during the study period. Data were not available for onset of disease.

³Receiver operating characteristic (ROC) area under the curve (AUC) for AGP predicting postpartum disease.

⁴Concentration offering the maximum difference between sensitivity and (1 − specificity).

⁵Negative association between plasma AGP and disease or culling.

⁶Insufficient sample size for diagnostic testing using ROC analysis (Hajian-Tilaki, 2014).

at 7 DIM and gradually declined to near prepartum concentrations by 21 DIM (Jafari et al., 2006). Bovine plasma AGP concentration is correlated with *AGP* mRNA abundance in the liver during the transition period, with an upregulation of hepatic *AGP* mRNA postpartum (McGuckin et al., 2020). Compared with plasma haptoglobin concentrations, AGP declines at a much slower rate postpartum (Sheldon et al., 2001; Jafari et al., 2006). In a study conducted by Horadagoda et al. (1999), cattle with chronic or acute inflammation were found to have serum AGP concentrations of 815 and 1,101 $\mu\text{g/mL}$, respectively, with a normal reference range of 200 to 450 $\mu\text{g/mL}$. Based upon the plasma AGP concentrations in the present study, most postpartum cows experienced elevated inflammatory tone but perhaps not chronic inflammation. However, great variation in AGP concentration occurs postpartum, so it is possible that a sizable minority of cows were experiencing chronic or even acute inflammation. We must also consider that the reference values in the study by Horadagoda et al. (1999) were determined by a single radial immunodiffusion assay, compared with an ELISA in our study, so direct comparisons are tenuous.

Associations Between AGP and DMI

One of the primary objectives of this study was to assess the relationship between AGP concentration and DMI. The negative associations between plasma AGP concentration and DMI were most significant postpartum, and AGP concentration on any given postpartum sampling day was negatively associated with DMI in subsequent weeks. This is consistent with previous work associating postpartum inflammatory markers with subsequent performance reduction in dairy cows (Huzzey et al., 2015; McCarthy et al., 2016). In pigs, serum AGP concentration is negatively correlated with daily feed intake and weight gain at 18 and 24 wk of age (Clapper et al., 2005), and activation of the immune system causing an increase in AGP tended to decrease daily feed intake in another study (Williams et al., 1997a,b). In fact, others have suggested that AGP may serve as an independent predictor of production performance impairment in livestock (Caperna et al., 2013).

Administration of exogenous human AGP reduces voluntary feed intake in rodents by acting on the hypothalamic leptin receptor and activating the JAK2/STAT3 pathway (Bellinger and Mendel, 1990; Sun et al., 2016), although very recent *in vitro* evidence suggests that bovine and human AGP do not elicit STAT3 signaling in cells expressing bovine leptin receptors (McGuckin et al., 2020). Another recent study administered purified bovine AGP both peripherally and centrally in sheep, with no effect on feeding rate or cumulative

intake (Gregg et al., 2019). It is unclear why human AGP activates the leptin receptor in mice but bovine and human AGP do not activate the bovine leptin receptor. It is certainly possible that species-specific differences in AGP modes of action exist. Further, plasma leptin concentration declines by $\sim 50\%$ in the transition to lactation and remains low postpartum (Liefers et al., 2003; Janovick et al., 2011) as energy balance is declining in dairy cows at the same time. Ruminant feed intake during periods of negative energy balance is generally refractory to leptin (Henry et al., 2001; Morrison et al., 2001; Ehrhardt et al., 2016); the same “leptin resistance” phenomenon may prevent AGP from having effects on downstream leptin receptor signaling in transition dairy cows.

Although we cannot determine the cause-and-effect relationships of AGP with DMI in the present study, it is intriguing that a negative association exists postpartum. Postpartum dairy cows experience elevated inflammation, and many factors are involved in an inflammatory response, including the release of cytokines, which have been implicated in modulating feed intake through hypothalamic action (Wong and Pinkney, 2004). To our knowledge, no other acute-phase proteins have been directly implicated in central control of feed intake, so it could be that a variety of molecules related to inflammation work centrally to affect intake during an inflammatory response.

To further evaluate the negative association between AGP and DMI, we calculated the predicted change in DMI for the d 3 AGP interquartile range (25th to 75th percentile) using parameter estimates from the best-fit equation for wk 1 DMI (Table 4). As d 3 AGP increased over the interquartile range, average daily DMI during wk 1 was calculated to decrease 8.5%, or 1.4 kg (Figure 2). For comparison, the reductions in DMI for retained placenta, metritis, and milk fever were estimated to be 0.8, 5.1, and 14.7 kg/d, respectively (Bareille et al., 2003). Although the reduction of DMI over the AGP interquartile range does not initially appear substantial compared with diseases that plague modern dairy operations, it is still concerning and warrants further investigation. Furthermore, weak forward associations suggest that AGP may serve as a leading indicator of suboptimal DMI, rather than a trailing indicator such as clinical disease diagnosis. For example, d 3 AGP was negatively associated with DMI beyond wk 1 (Table 4), and AGP on d 7 and 14 was negatively associated with DMI for multiple weeks. One limitation of these data is the relatively high interplate variability (CV of 17.3%) for AGP quantification, which may in part explain the modest associations between AGP and DMI. A more precise measurement of AGP might have better explained variation in DMI postpartum.

Prognostic Ability of AGP to Predict Poor DMI

The fact that AGP may serve as a leading indicator of suboptimal postpartum DMI led us to our third objective, which was to assess the prognostic value of AGP to predict cows at risk of low DMI using ROC analysis. The ROC analysis is a method for evaluation of the accuracy of a clinical diagnostic test, where the optimum combination of sensitivity (true positive) and specificity (lack of false positive) of the test are maximized in ideal situations (Gardner and Greiner, 2006). As a continuous variable, DMI presents challenges for ROC analysis due to the difficulty in selecting a useful cutoff point (Gardner and Greiner, 2006). For determination of low DMI in this study, considering that there has been no proposed threshold of acute DMI depression in transition cows, we used the arbitrary definition of wk 1 DMI that is 1 SD below the mean. In the current study, the AUC of 0.6 (Figure 3) does not create confidence in the ability of AGP to predict acute depressed DMI. Constraining the definition of low DMI to 2 SD below the mean did not improve diagnostic performance (AUC = 0.55; data not shown). Therefore, although AGP is clearly negatively associated with DMI postpartum, it is a poor predictor of acute DMI depression.

Relationship Between AGP and Common Biomarkers

Should AGP be a useful indicator of future DMI depression, it would ideally be independent of other blood biomarkers that are cheaply and easily measured today, such as glucose or BHB. Out of 107 measured associations in this study between AGP and other blood biomarkers throughout the peripartum period (Supplemental Tables S1 to S8, <https://doi.org/10.3168/jds.2020-19025>), only 8 were significant. Considering the number of analyses for these comparisons, the 8 significant results may be a function of type I error at the 95% confidence rate. The finding that AGP is poorly associated with other biomarkers at many time points suggests that it could serve as an independent biomarker for predicting postpartum health or performance.

Despite minimal associations between AGP and plasma biomarkers, we detected several associations that may be biologically meaningful. The positive association between AGP and BHB on d 7 (Table 5) occurs at a time when both factors are normally increasing due to calving and the induction of negative energy balance. Others have demonstrated an association between acute-phase proteins and ketosis in postpartum cows (Abuajamieh et al., 2016), and more controlled studies have administered inflammatory components, such as tumor necrosis factor- α , and increased liver fat

concentration (Bradford et al., 2009), BHB (Trevisi et al., 2009), and incidence of ketosis (Yuan et al., 2013). In this light, the limited associations between AGP and BHB concentrations postpartum are biologically plausible.

Not surprisingly, plasma AGP concentration was positively associated with plasma haptoglobin concentration on d 3, d 7, and d 14 (Table 5). Postpartum AGP and haptoglobin have been shown in other studies to follow a similar pattern postpartum with a spike on d 7 before declining (Jafari et al., 2006), but the haptoglobin concentration declines to stable concentrations about 1 wk earlier than does AGP (Sheldon et al., 2001). This may explain the lack of persistent future associations between AGP and haptoglobin in this study despite the strong associations on the same day, and may reflect the anti-inflammatory properties of AGP and its function to resolve inflammation (Ceciliani and Lecchi, 2019). Conversely, AGP and haptoglobin have an extremely weak correlation in healthy pigs (Clapperton et al., 2007). Others have shown a tendency for a relationship between prepartum haptoglobin and postpartum disease incidence (Huzzey et al., 2011), and peripartum haptoglobin is negatively associated with milk production (Huzzey et al., 2015).

Relationship and Prognostic Ability of AGP for Disease and Culling

Our final objective was to assess the prognostic value of AGP to predict postpartum disease. In this study, postpartum AGP concentration was positively associated with incidence of retained placenta and metritis, but for variables with sufficient sample size the diagnostic ability was marginal based upon the ROC AUC (Table 6). These diseases are common for postpartum dairy cows (Dubuc and Denis-Robichaud, 2017). Despite this strong positive association between AGP and postpartum disease, it is unlikely that AGP is a predictor of disease, but, rather, AGP may be increasing with other positive acute-phase proteins in response to the disease insult (Horadagoda et al., 1999; Sheldon et al., 2001). In normal uterine defense systems postpartum, bacterial invasion causes immune cell infiltration and inflammation, which can persist with the pathogenesis of endometritis and metritis (Esposito et al., 2014). Unfortunately, lack of data prevented us from further analyzing onset of disease and its relationship with AGP concentration in transition cows. Incidence of disease for the data set is outlined in Table 7.

We were surprised to observe that AGP on d 1 was associated with reduced culling through 280 DIM. Although inflammation can be associated with negative production outcomes in chronic instances, it may be

Table 7. Total incidence of postpartum disease and survival to 280 DIM by study in transition dairy cows

Item	n	
	Total cases	Total animals ¹
Hyperketonemia	94	377
Retained placenta	41	377
Metritis ^{2,3}	41	318
Mastitis	52	377
Culled by 280 DIM	69	371

¹No disease incidence data were available from Carpenter et al. (2018).

²Data on metritis incidence were not available from Olagaray et al. (2019).

³Defined as fetid, watery discharge. Complete descriptions can be found in the respective publications (Leno et al., 2017a,b; Zenobi et al., 2018; Bollatti et al., 2020).

required as a part of the natural adaptive mechanisms for a successful transition to lactation (Bradford et al., 2015). Transgenic overexpression of AGP increased survivability from lethal *Klebsiella pneumonia* infection in mice, and administration of AGP in normal animals before infection also had dramatic nonspecific protective effects (Hocheppied et al., 2000). Similarly, administration of AGP before a lethal dose of tumor necrosis factor- α prevented death (Libert et al., 1994). The cows in this study were generally not exposed to lethal quantities of harmful pathogens or cytokine storms, and their survivability to 280 d is much longer than those found in mouse studies. However, we could hypothesize that the elevated AGP concentrations as a result of the natural increase in inflammation postpartum had some protective effects upon subsequent pathogen exposure, considering postpartum cows have elevated susceptibility to infection. Nonetheless, despite this negative association, the diagnostic ability of d 1 AGP to predict culling, as determined by ROC analysis, was poor (AUC = 0.63; Table 6).

CONCLUSIONS

Plasma AGP concentration significantly increases by d 3 postpartum, with an apparent peak at 14 DIM. We found that AGP was negatively correlated with DMI postpartum, and DMI decreased approximately 8.5% as plasma AGP increased over the interquartile range. It is possible that AGP could be used as a measure of inflammatory status in transition dairy cows, and an ELISA can serve as a reliable and repeatable method of quantification. We found that AGP was not related to many other blood biomarkers except for haptoglobin, demonstrating independence from other common biomarkers commonly used today. Although we detected a strong association between AGP and transition diseases, the disease may be causing the increase in AGP

instead of AGP predicting disease. However, based upon ROC analysis, the diagnostic ability of AGP to predict suboptimal DMI is poor. Further work should focus on in vivo AGP administration to determine effects on feeding behavior, and work to determine potential mechanisms of AGP action on the hypothalamus in ruminant models should be conducted.

ACKNOWLEDGMENTS

This work was carried out with support from the Animal Nutrition, Growth, and Lactation program (grant no. 017-67015-26491) and Hatch Project 1018048 from the USDA National Institute of Food and Agriculture (Washington, DC). The authors thank Chadron Koehn and Gary Davis (Kansas State University, Manhattan) for many hours spent performing countless ELISA experiments. Their attention to detail and accuracy is greatly appreciated. The authors have not stated any conflicts of interest.

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